The chemical composition and sensory properties of cooked beef aroma

Galt, A M

Fig:20:Initial Blank Oven Chromatogram - Expt.3a(i)

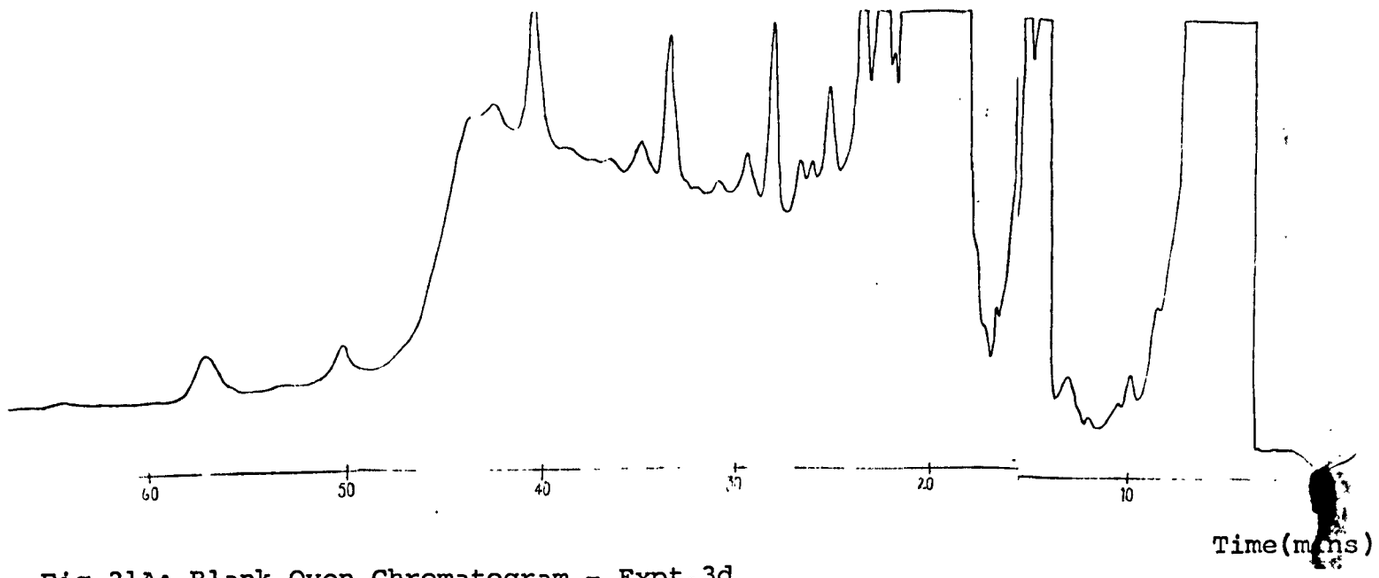


Fig.21A: Blank Oven Chromatogram - Expt.3d

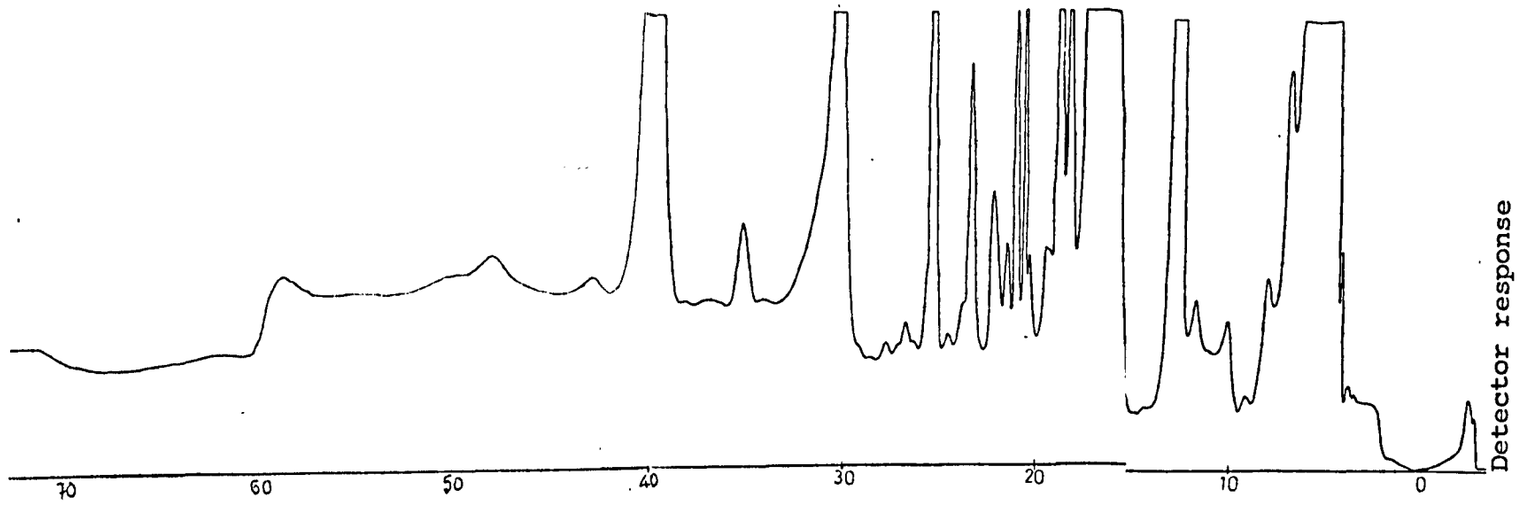


Fig.21B: Vent Tube Chromatogram - Expt.3d

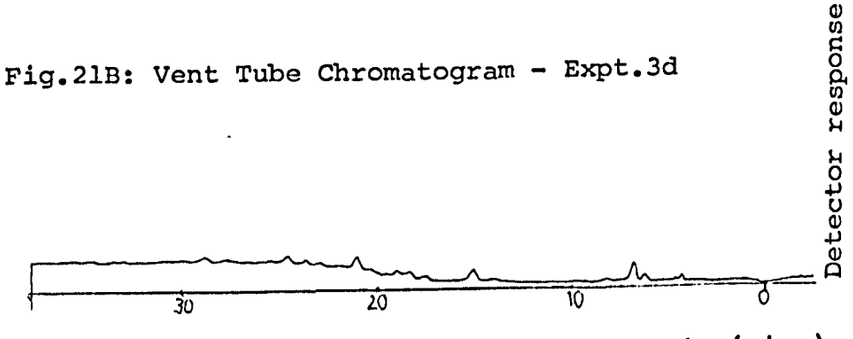


Fig.22A: Chromatogram showing N_2 Contamination - Expt.3e(ii)

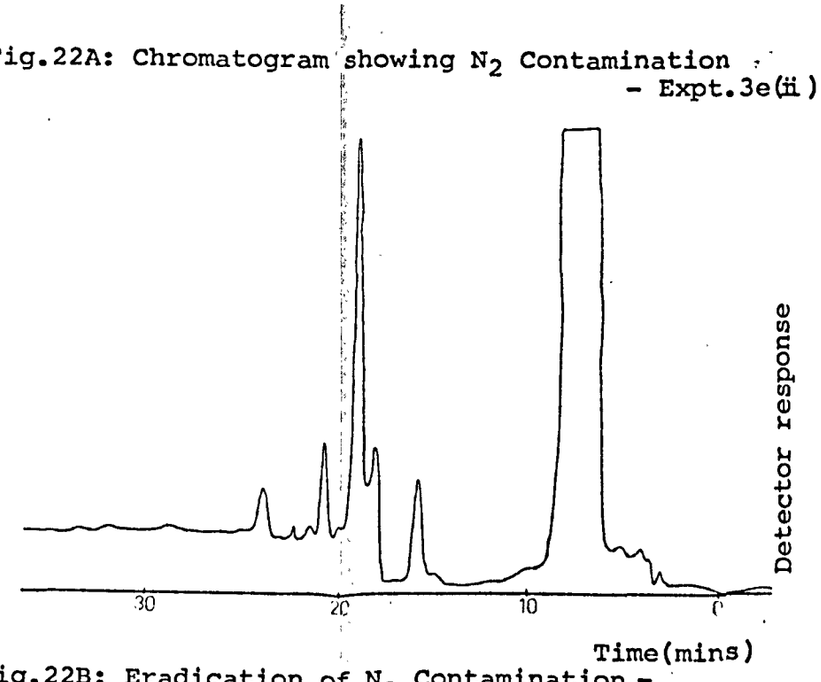


Fig.22B: Eradication of N_2 Contamination - Expt.3e(iii)

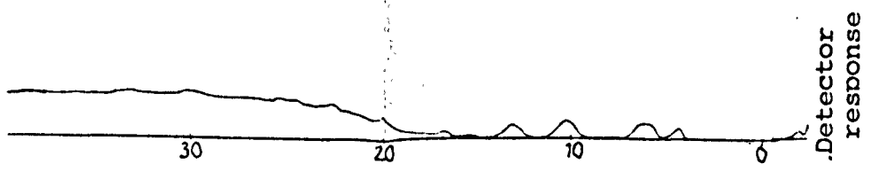


Fig.22C: 5min. HP Air Sample at Ambient-Expt.3e(iv)

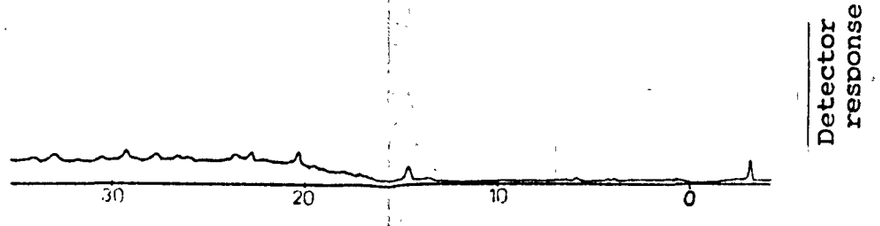


Fig.22D: 5min. HP Air Sample Taken via Oven - Expt.3e(v)

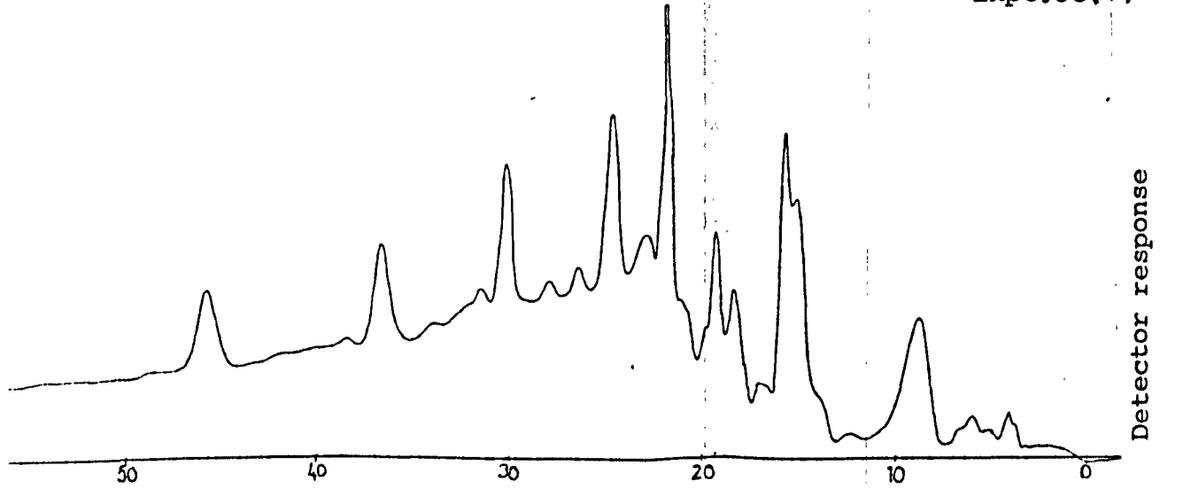


Table 17 : Results of Unrestricted Factor Analysis Performed on 33 Odour Qualities

FACTOR 1		FACTOR 2		FACTOR 3		FACTOR 4		FACTOR 5	
OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading
sickly*	0.816	savoury*	0.829	meaty-raw	0.715	vegetables overcooked	0.881	throaty*	0.801
blood-like*	0.769	Bovril*	0.688	Marmite-like	0.662	cooked cabbage*	0.802	sharp/pungent*	0.745
nasty smelling*	0.687	meaty-roast*	0.655	broth-like*	0.617	cool/cooling*	0.446	herbal/hay	0.418
earthy/soil*	0.637	odour strength*	0.620	meaty-boiled	0.509	sweet	0.424	sweaty	0.292
sweaty*	0.581	preference	0.461	Bovril-like	0.386	toasted	0.306		
animal/goaty*	0.562	broth-like	0.425	flat/dull	0.373	blood-like	0.259		
cool/cooling*	0.561	animal/goaty	0.311						
oily/fatty*	0.466								
musty/mouldy*	0.458								
meaty-boiled	0.421								
sweet	0.310								
meaty-raw	0.305								
% Variance Explained	14.6		6.8		7.8		6.9		6.0
Cumulative % Variance Explained	14.6		25.4		33.1		40.9		46.0
NEGATIVE FACTOR LOADINGS	NONE	musty/mouldy	-0.323	NONE		animal/goaty	-0.260	buttery	-0.412
		buttery	-0.315					preference	-0.273
		flat/dull	-0.298						

FACTOR 6		FACTOR 7		FACTOR 8		FACTOR 9		FACTOR 10	
OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading
fragrant*	0.761	burnt*	0.785	aromatic*	0.817	paint-like*	0.719	ammonia-like*	0.790
spicy*	0.748	toasted*	0.491	oily/fatty*	0.577	nasty smelling	0.390	buttery	0.422
herbal/hay	0.429	odour strength*	0.478	toasted	0.397	oily/fatty	0.284	earthy/soil	0.310
sweaty	0.351	musty/mouldy	0.427	earthy/soil	0.326			toasted	0.309
preference	0.351	meaty/roast	0.311					animal/goaty	0.307
Marmite-like	0.253							sharp/pungent	0.305
% Variance Explained	5.5		4.6		4.4		3.6		3.5
Cumulative % Variance Explained	51.5		56.1		60.5		64.1		67.6
NEGATIVE FACTOR LOADINGS	NONE	herbal/hay	-0.345	Bovril-like	-0.329	preference*	-0.459	NONE	
		flat/dull	-0.329	animal/goaty	-0.268	buttery	-0.369		
		meaty/boiled	-0.273						

Table 18 : Results of Unrestricted Factor Analysis Performed on 24 Odour Qualities.

FACTOR 1		FACTOR 2		FACTOR 3		FACTOR 4		FACTOR 5	
OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading
broth-like*	0.851	cooked cabbage*	0.855	toasted*	0.817	ammonia-like*	0.807	oily/fatty	0.820
meaty-boiled*	0.711	overcooked vegetable*	0.810	burnt*	0.743	animal/goaty*	0.589	paint-like	0.660
				meaty-roast*	0.602	sharp/pungent*	0.571	earthy/soil	0.435
meaty-roast*	0.541	cool/cooling*	0.598			earthy/soil*	0.534	blood-like	0.402
meaty-raw*	0.469	blood-like	0.423					cool/cooling	0.358
blood-like	0.439	sweat	0.401						
animal/goaty	0.424	meaty-boiled	0.311						
% Variance Explained	15.8		10.7		8.7		7.8		6.9
Cumulative % Variance Explained	15.8		26.5		35.2		43.0		49.9

FACTOR 6		FACTOR 7		FACTOR 8		FACTOR 9	
OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading	OQ	Factor Loading
spicy*	0.763	musty/mouldy*	0.812	flat/dull*	0.754	buttery*	0.868
fragrant*	0.752	animal/goaty	0.396	meaty-raw*	0.593	sweet	0.258
sweaty*	0.513	sweaty	0.380	herbal/hay*	0.490		
herbal/hay	0.416	blood-like	0.364				
		meaty-raw	0.286				
		earthy/soil	0.285				
% Variance Explained	5.5		4.9		4.7		4.4
Cumulative % Variance Explained	55.4		60.3		65.0		69.4

Footnote: * represents OQ with significant factor loading.

Table 27 : Chemical compounds and OPA's (Expt. 15) in heat desorption period fractions.

PEAK DIFFERENCES	
Pre-Flush/Heating > Flush-on	Flush-on > Pre-flush/Heating
1. air	
2. carbon dioxide	
3. pentane - chemical solvent	
4. hexamethyldisiloxane-chemical solvent	
5. heptane	
6. acetaldehyde - musty, faecal, sulphurous	
7. trimethylethoxysilane - dry cleaning solvent 3-methylpentane - sweet, estery, chemical solvent, ether	
8. methanethiol - rotten vegetables, faecal, bad eggs	
9. a hydrocarbon (?methylpropane) - fruity, fragrant, acetaldehyde) cut green leaves	
10. ? - sickly, sweaty, rancid, faecal, meaty boiled	
11. ? - cooked vegetables, canned sweetcorn	
12. ? - chemical solvent, H_2S, burnt, meaty	
13. ? - musty, mouldy, oily, fatty, cooked cabbage	
14. ? - sweet, buttery, popcorn	
(15) acetone + carbon disulphide - chemical solvent, metallic	
(16) ? - roast pork, baked breadcrusts	
(17) dimethylsulphide - faint warm rubber, meaty ←buttery, caramel, creamy, butterscotch ←meaty savoury, beef, gravy, pleasant.	
(20) ?) 21. ?) chemical solvent, formalin, resinous 22. ?)	
23. a C_4 amine MW73 - meaty roast	
(24) propanal + methylpropanal - chemical solvent formalin	
meaty (25) methanol	
(26) ethanol + 1,1,1-trichloroethane	
(27) 3-methylbutanal - toasted bread, meaty sweet	
(28) H_2O	
(29) diacetyl - buttery, caramel, toffee, candyfloss	
(33) but-2-en-1-al- green sap, woody, fragrant	
(40) a methylpentanolactone - estery, floral(violets) fragrant, fruity	
(47) 3-hydroxybutanone (acetoin) - sweet, baked cakes	
	49. ? - burnt, charred, meaty, nutty, spicy, unpleasant
	51. ? - musty, mouldy
	52. hept-3-ene- fruity, fragrant, sweet, floral, nutty
	53. ? - coconut, green, floral
	54. ? - sickly, meaty, sweaty, charred beef, earthy, charred, burnt, roasted, meaty, unpleasant, Marmite.
	55. ? - burnt milk, baked cakes, buttery, toffee
	56. ? - green, wet grass, green pepper, cucumber, cured meat, meaty
	57. dimethyltrisulphide - smoky, chemical solvent, sweet, fruity, perfume meaty, Oxo, oily, fatty, musty, mouldy, roast beef
	58. ? - meaty, pork, burnt rubber, roasted potatoes
	59. ? - charred, roasted nuts, green peppers
	60. ? - Bovril, charred, smoky, burnt, beef meaty, musty, mouldy
	61. octan-1-ol (?) - buttery, nutty cooked meat, Oxo
	62. ? - cucumber, green peppers
	63. benzaldehyde - nutty, almonds, fragrant, oily, fatty green pepper, charred, smoky
	64. ? - green peppers, roast potatoes
	65. ?

(i) brackets around peak numbers in the first column indicate peaks also present in the sample collected during the flush-on stage of heat desorption, whereas unbracketed peaks were absent in that sample.

(ii) OPA's which are underlined highlight the sensory properties which differentiated significantly between these two samples in the sensory analysis Expt. 12.

Fig.7: Blank Tenax Chromatogram - Expt.1a

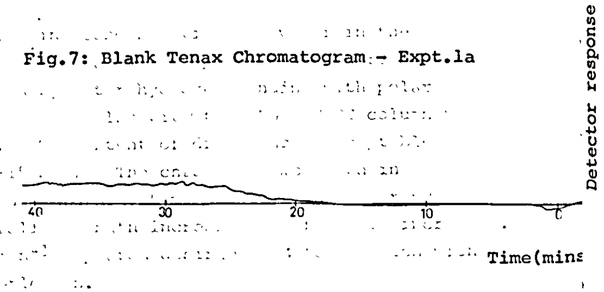


Fig.8: Initial Aroma Isolate - Expt.1b

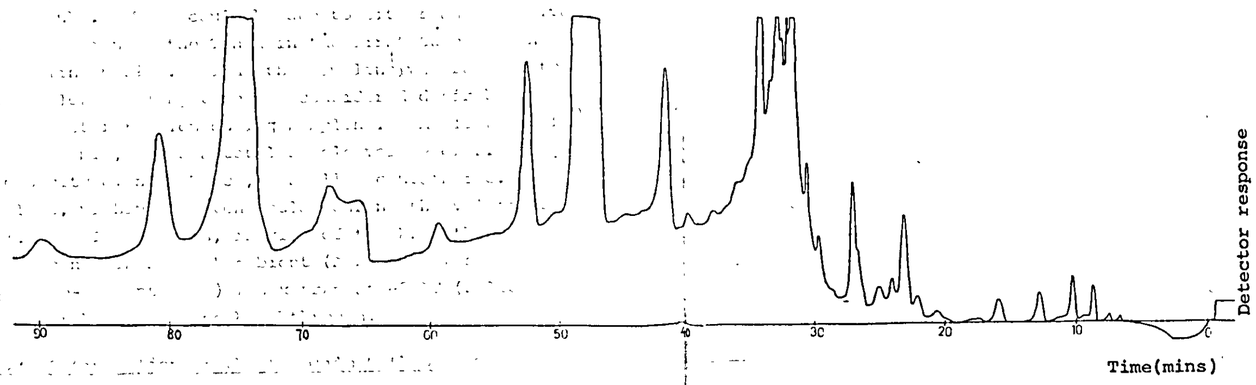


Fig. 8 cont'd

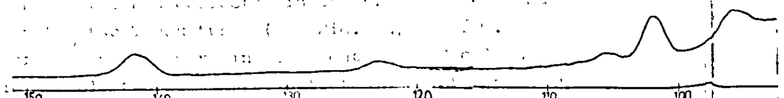


Fig.9: Blank Oven Chromatogram - Expt.1c

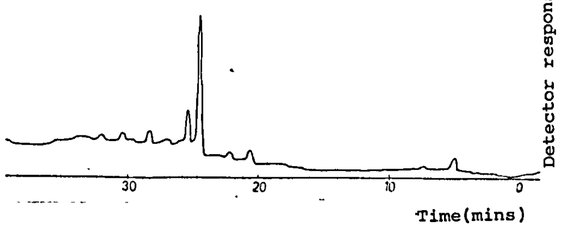


Fig.10A: Aroma Isolate (100 ml min^{-1}/ 15 min) - Expt.1d

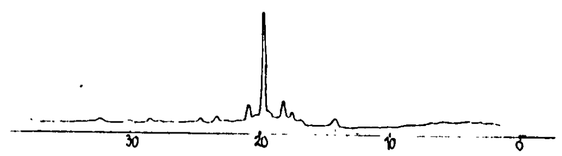


Fig.10B: Aroma Isolate (125 ml min^{-1}/ 15min.) - Expt.1d

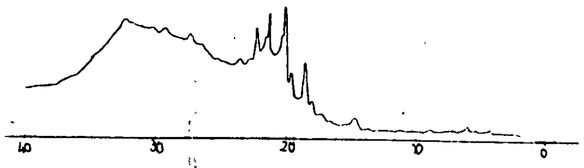


Fig.10C: Aroma Isolate(175 ml min^{-1}/ 15min.) - Expt.1d

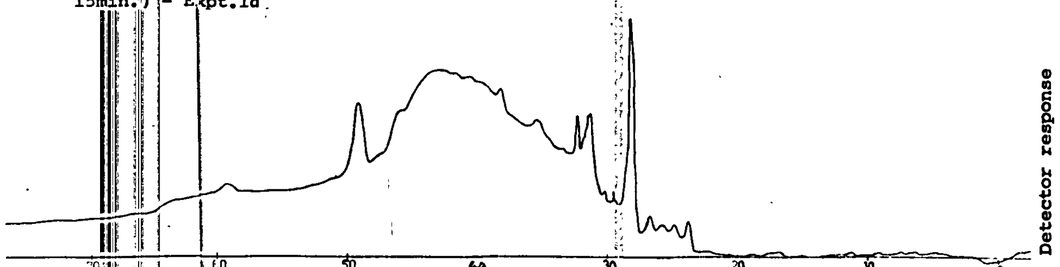


Fig.10D: Aroma Isolate(250ml min^{-1}/15min.) - Expt.1d

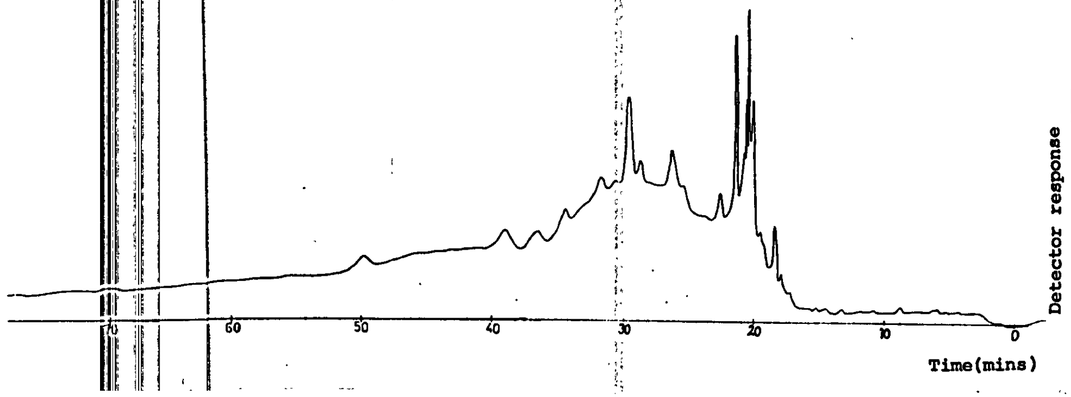


Fig.11A: Amb Chromatogram – Expt.2b

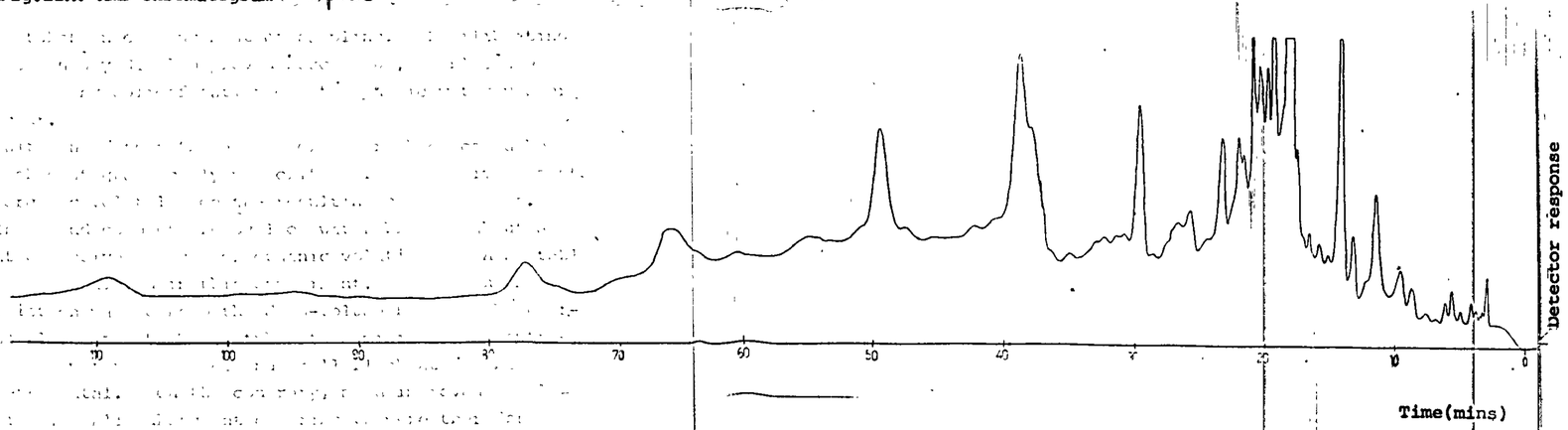


Fig.11B: Sba Chromatogram

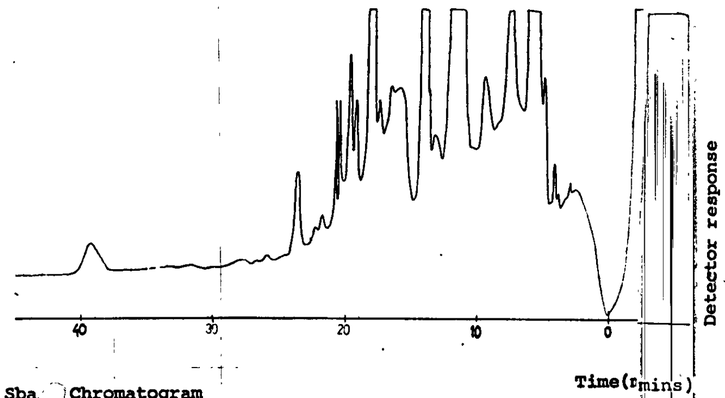


Fig.12B: Sba Chromatogram

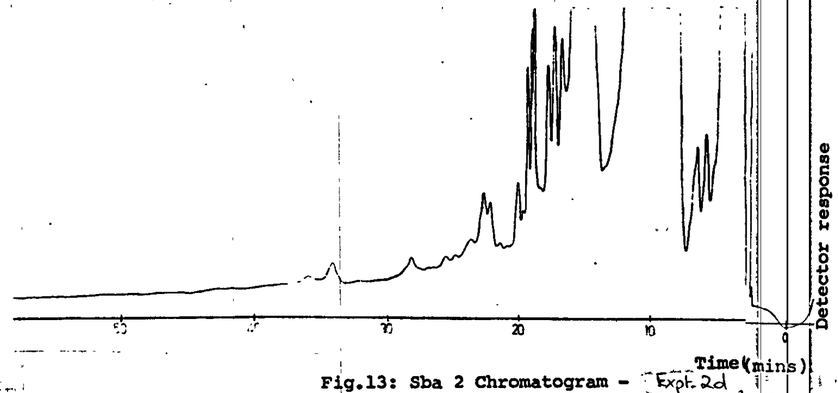


Fig.12A: Amb Chromatogram – Expt.2c

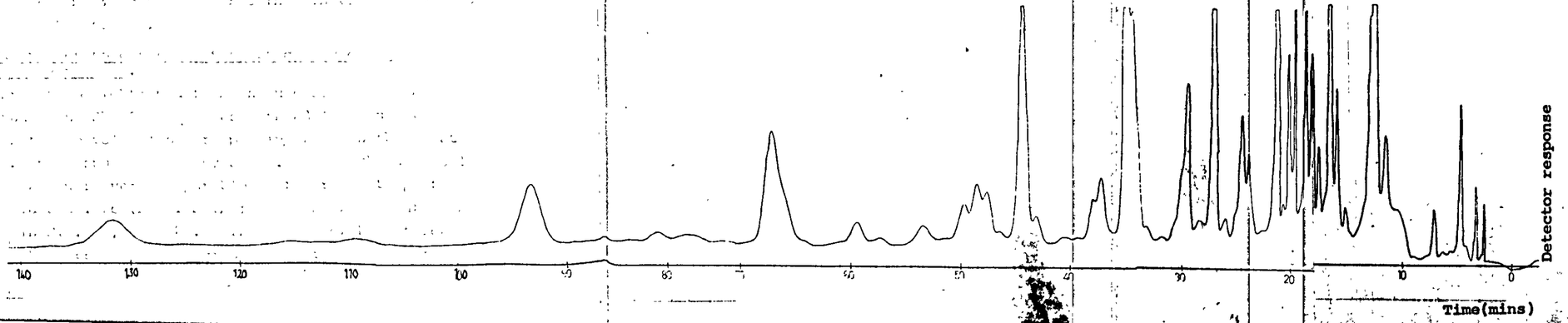


Fig.13: Sba 2 Chromatogram – Expt.2d

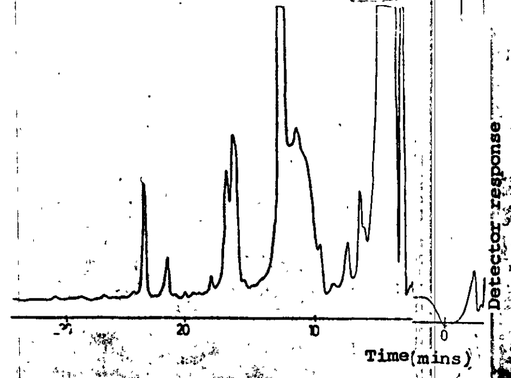


Fig.14A: Amb Chromatogram(500ml min^{-1}/10 min.)-Expt.2e(i)

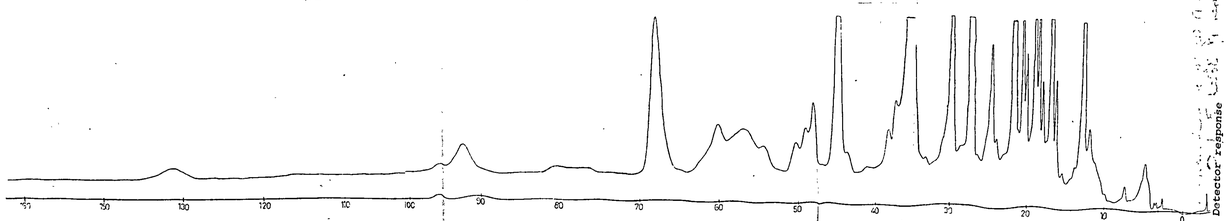


Fig.15A: Amb Chromatogram(250ml min-1/5 min.)-Expt.2e(ii)

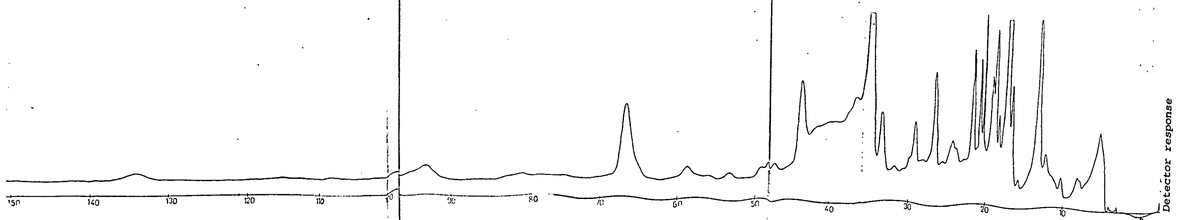


Fig.16A: Amb Chromatogram(250ml min^{-1}/10 min.-Expt.2e(iii)

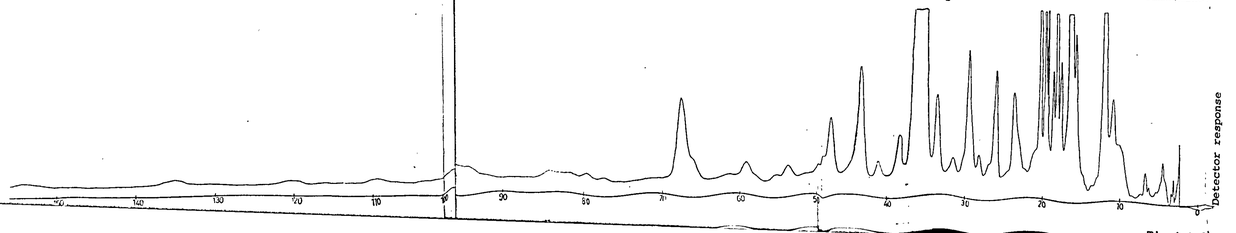


Fig.14B: Sba 1 Chromatogram - Expt. 2e(i)

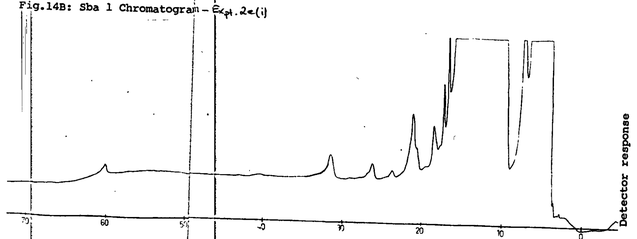


Fig.15B: Sba 1 Chromatogram - Expt. 2e(ii)

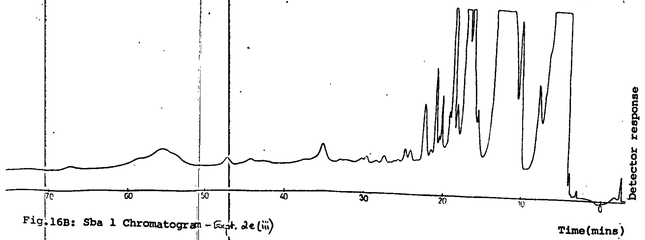


Fig.16B: Sba 1 Chromatogram - Expt. 2e(iii)

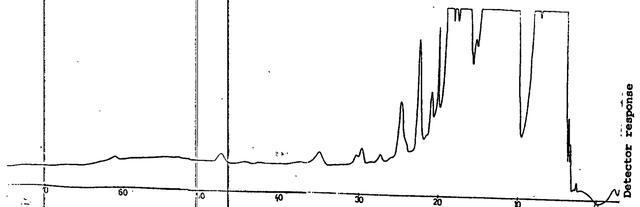


Fig.14C: Sba 2 Chromatogram - Expt. 2e(i)

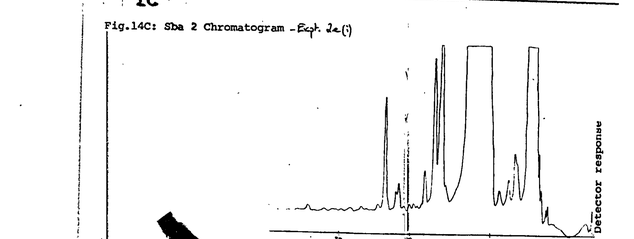


Fig.15C: Sba 2 Chromatogram - Expt. 2e(ii)

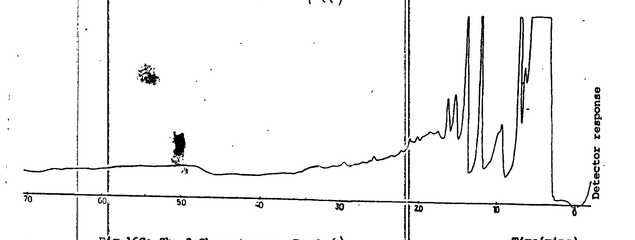


Fig.16C: Sba 2 Chromatogram - Expt. 2e(iii)

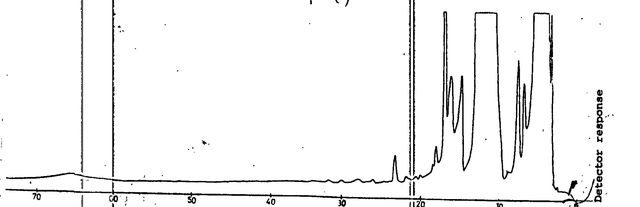


Fig.17A: Sba 1 Chromatogram - Expt.2f

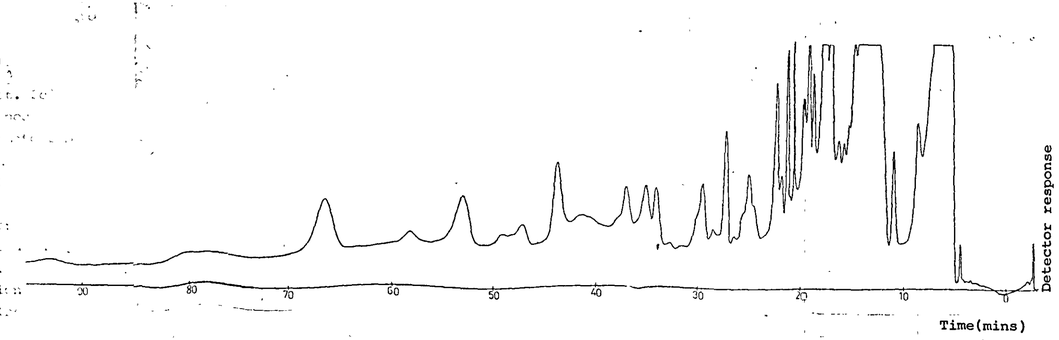


Fig.17B: Sba 2 Chromatogram - Expt.2f.

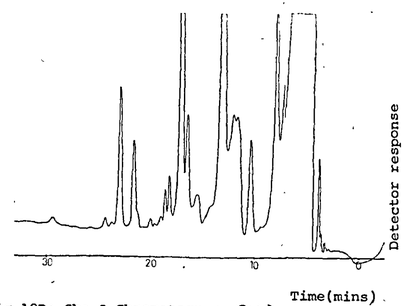


Fig.18A: Sba 1 Chromatogram using '4 X S' System - Expt.2g

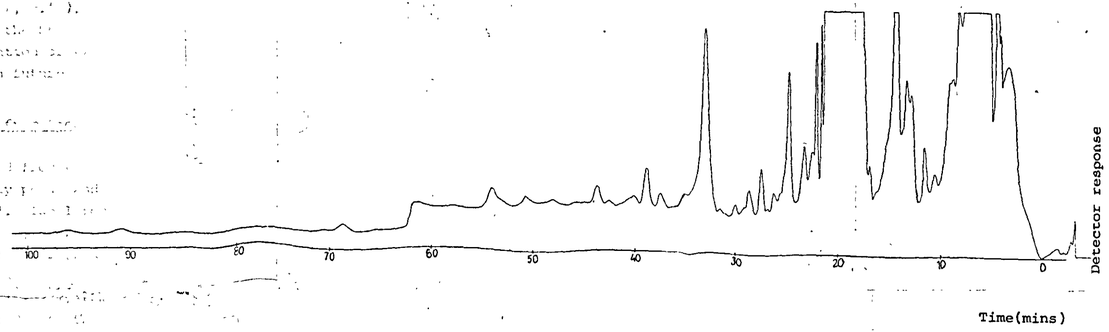


Fig.18B: Sba 2 Chromatogram - Expt.2g

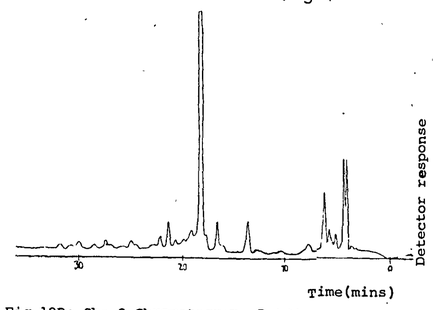


Fig.19A: Sba 1 Chromatogram - Expt.2h

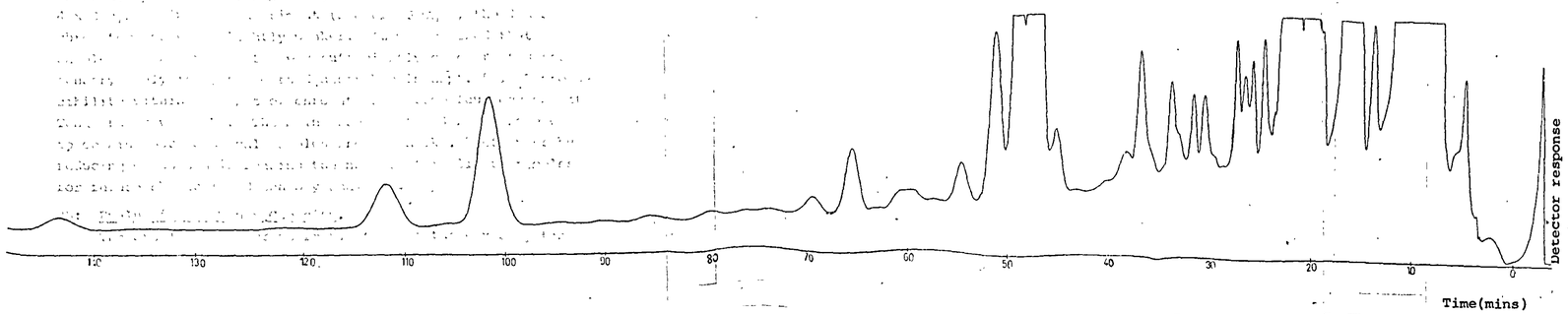


Fig.19B: Sba 2 Chromatogram - Expt.2h

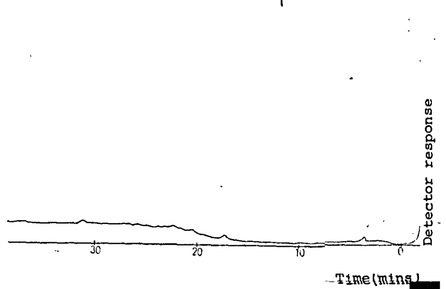


Fig.17A: Sba 1 Chromatogram - Expt.2f

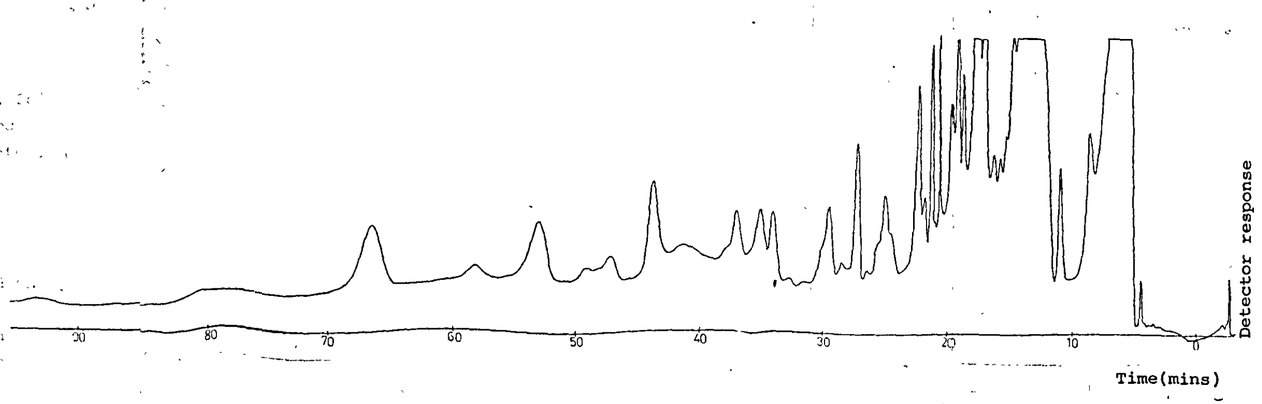


Fig.17B: Sba 2 Chromatogram - Expt.2f

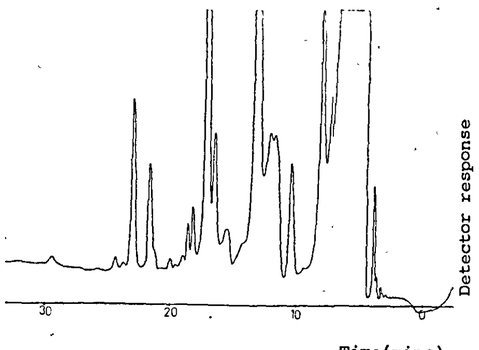


Fig.18A: Sba 1 Chromatogram using '4 X S' System - Expt.2g

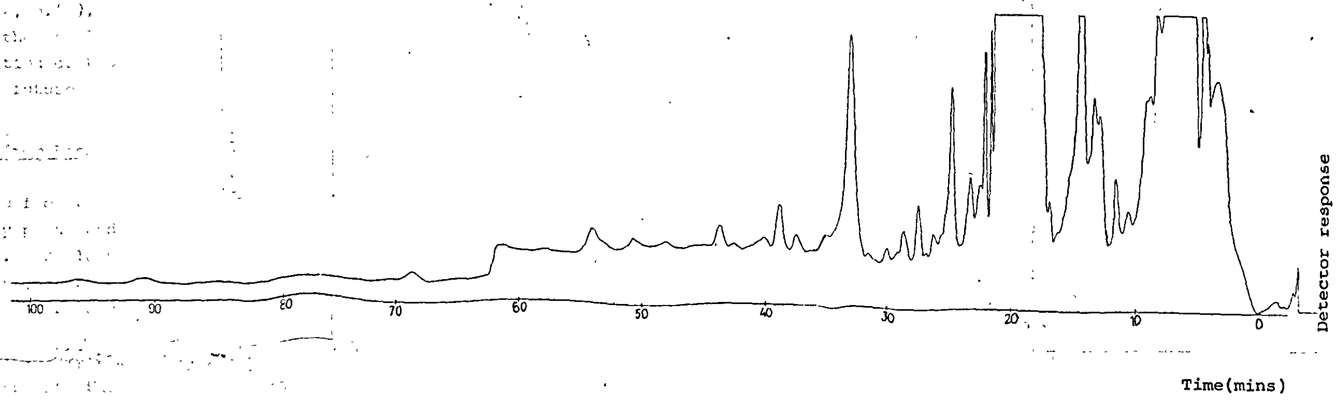


Fig.18B: Sba 2 Chromatogram - Expt.2g

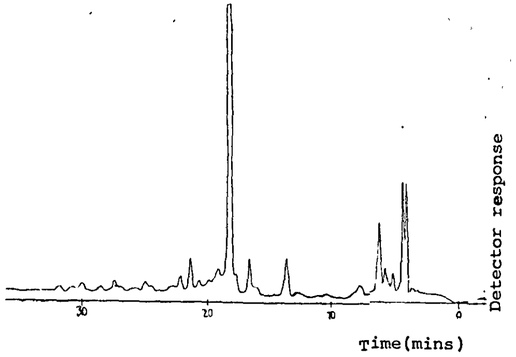


Fig.19A: Sba 1 Chromatogram - Expt.2h

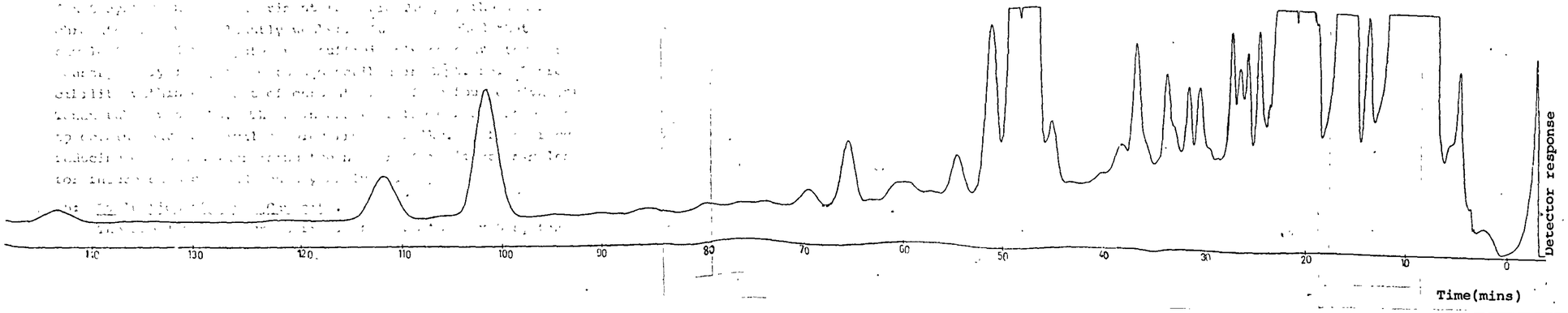


Fig.19B: Sba 2 Chromatogram - Expt.2h

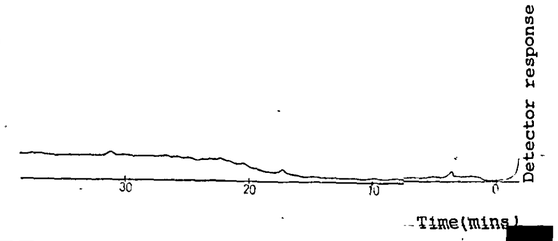


Fig.23: Blank Oven Chromatogram using Teflon Bag- Expt.3f(i)

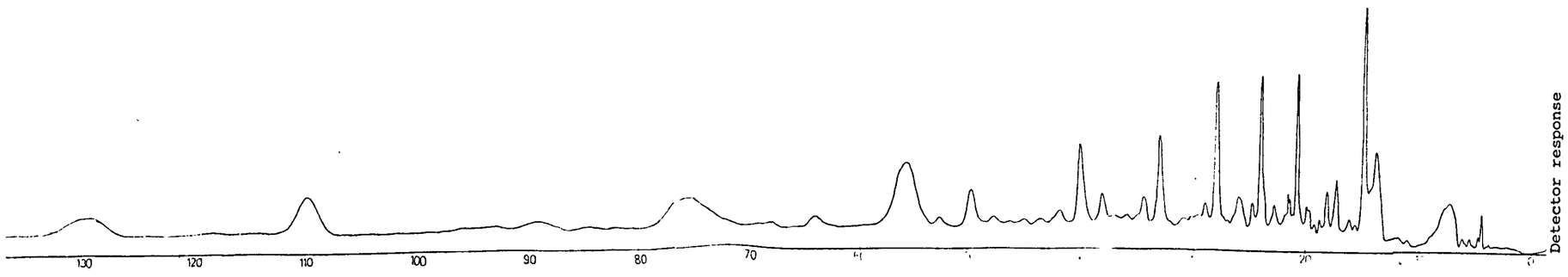


Fig.25: Blank Oven Chromatogram with Optimised Flask 'Flushing' Procedure - Expt.3h(i)

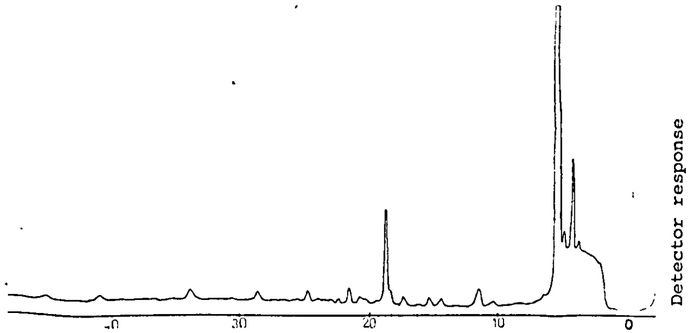


Fig.24A: Blank Oven Chromatogram of Silylated Flask - Expt.3g(ii)

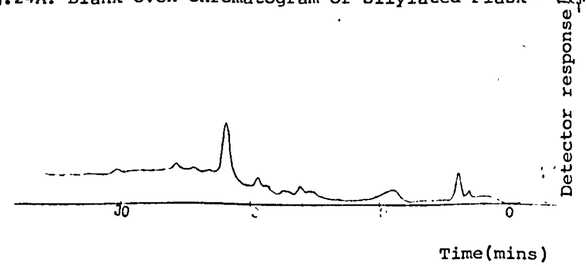


Fig.24B:Blank Oven Chromatogram of Re-silylated Flask - Expt.3g(iii)

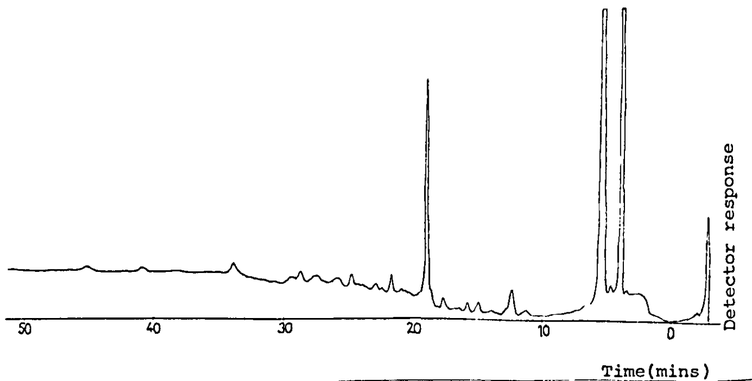


Fig.26A: External Vent TubeChromatogram-Expt.3i(i)

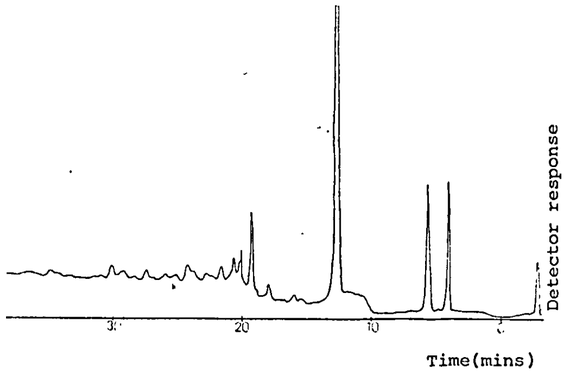


Fig.26B: Blank Oven Chromatogram with External Vent Tube for Heating Only - Expt.3i(i)

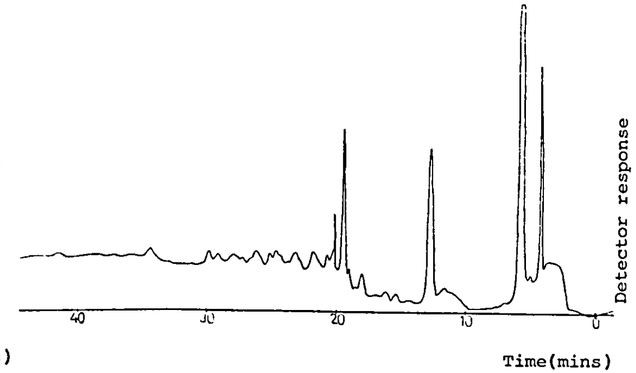


Fig.26C: Blank Oven Chromatogram from Venting via External Vent Tube- Expt.3i(ii)

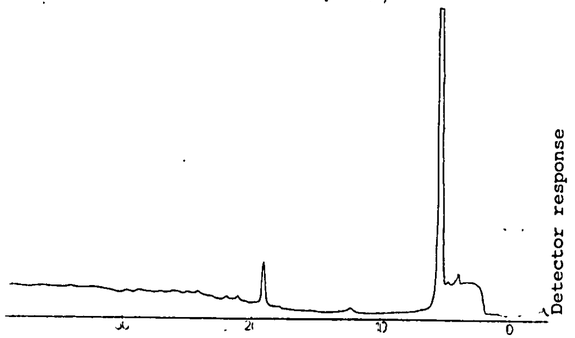


Fig.26D: Blank Oven Chromatogram from Venting via the Sample Tenax Tubes - Expt.3i(iii)

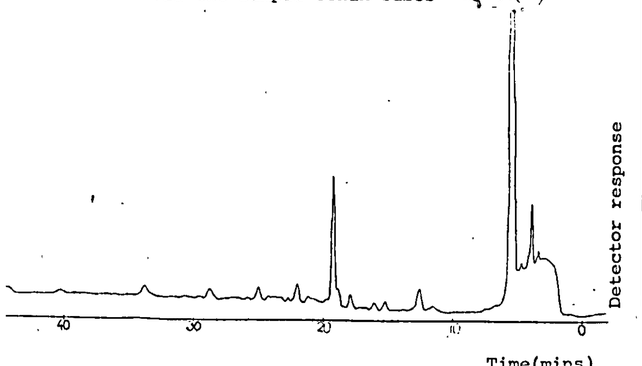


Fig.27A: 1h Aroma Isolate -Attenuation 5 x 10^2 - Expt.4b.

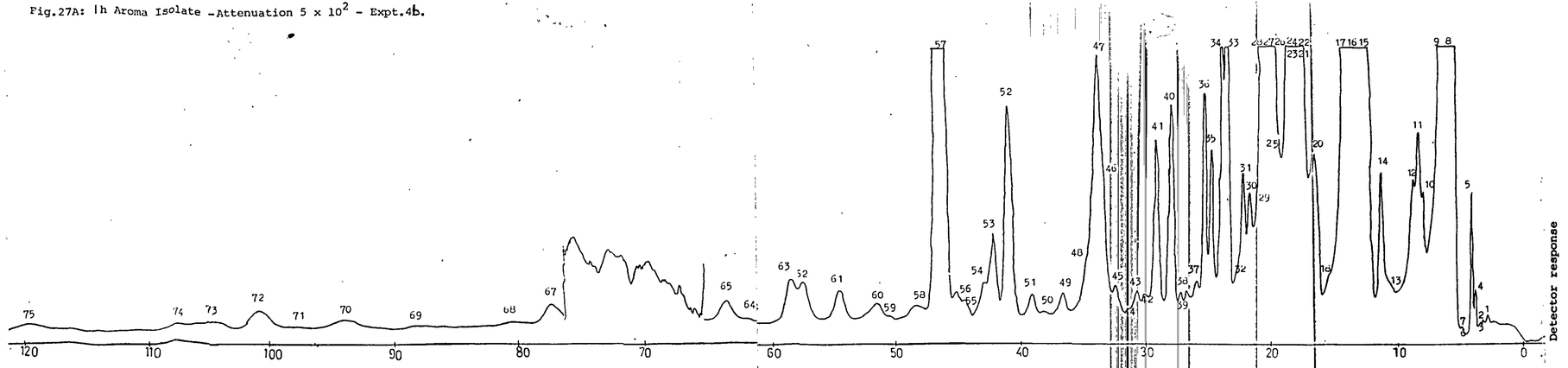


Fig.27B: 1h Aroma Isolate - Att^n. 1 x 10^3

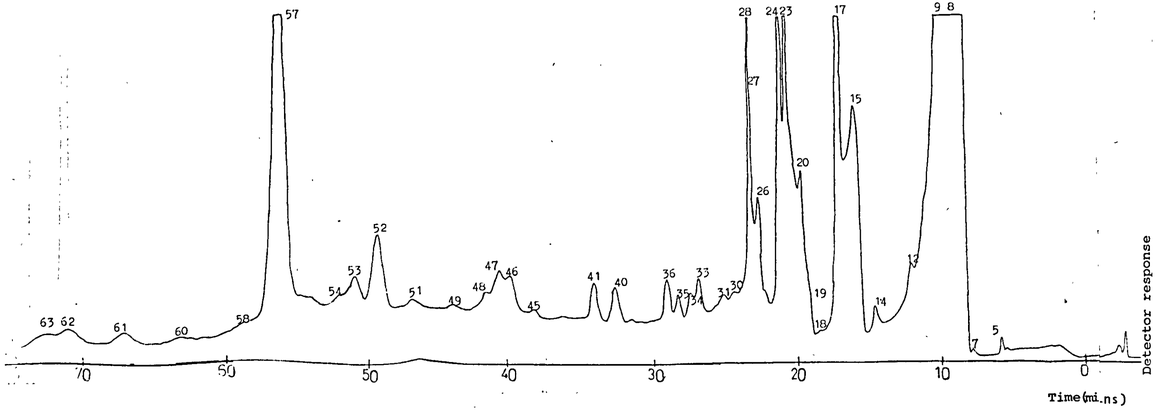


Fig.27C: 1h Aroma Isolate - Att^n. 2 x 10^3.

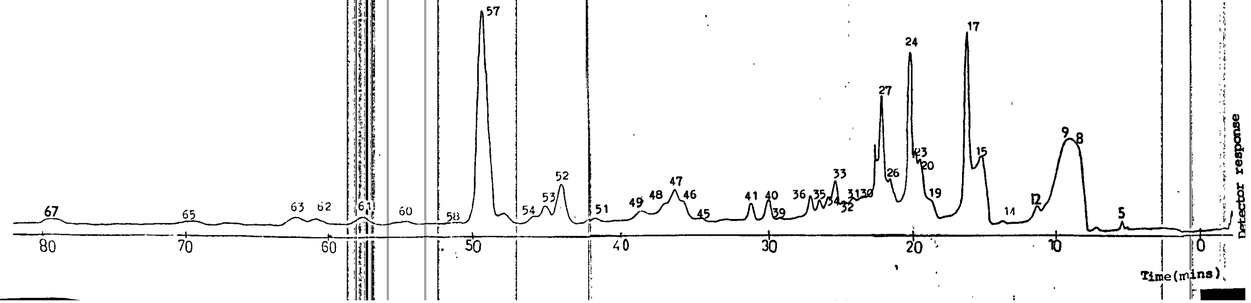


Fig.28A: 4h Aroma Isolate - Attenuation 5 x 10^2. - Expt.4c

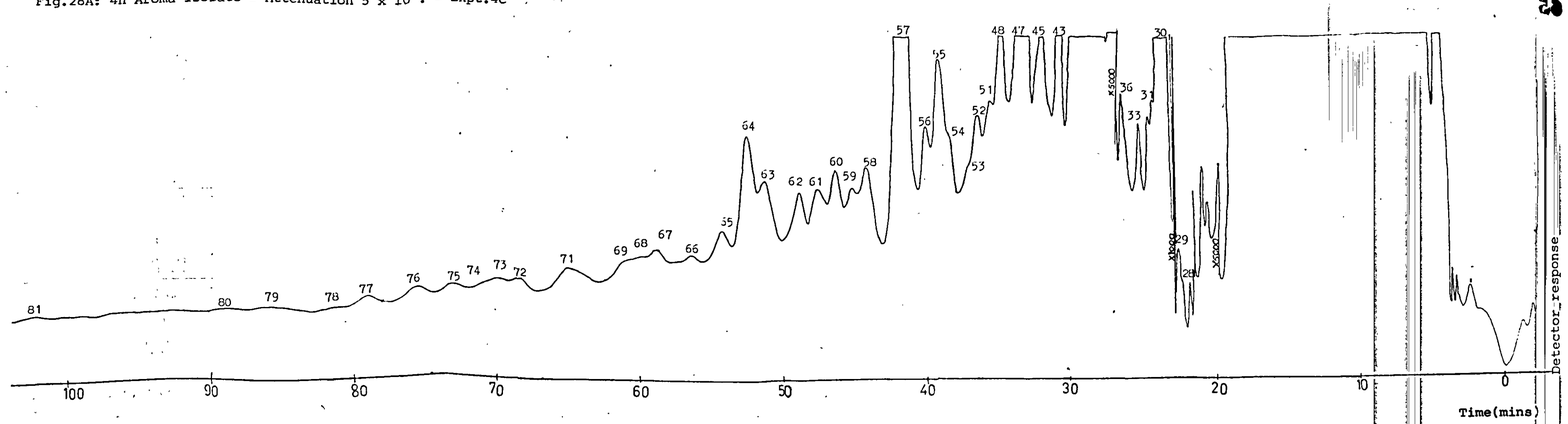


Fig.28B: 4h Aroma Isolate - Att^n. 2 x 10^3.

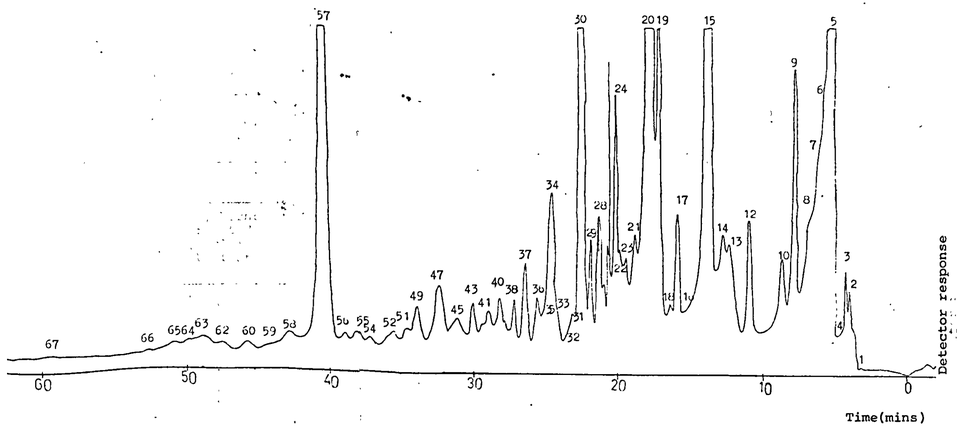


Fig.28C: 4h Aroma Isolate - Att^n. 5 x 10^3.

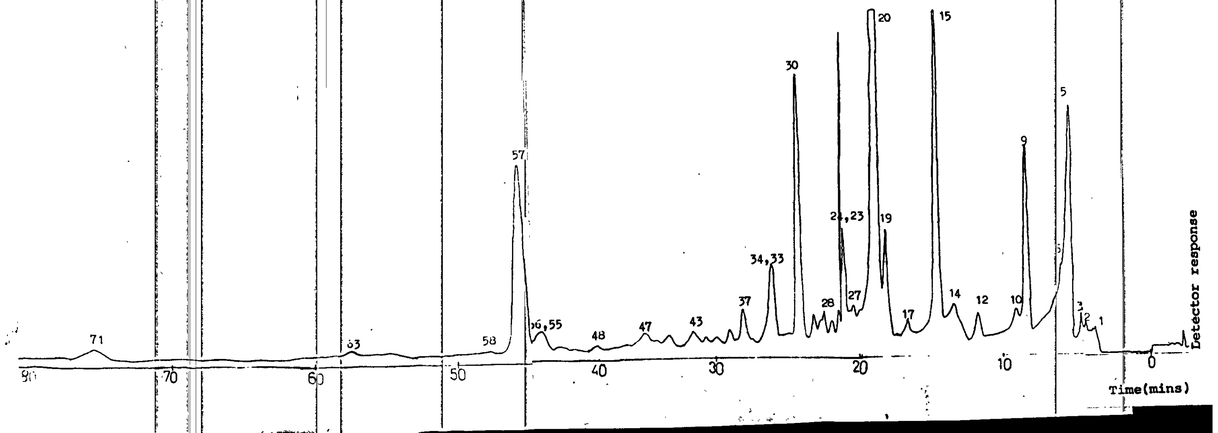


Fig.28D: 4h Aroma Isolate - Att^n 2 x 10^4.

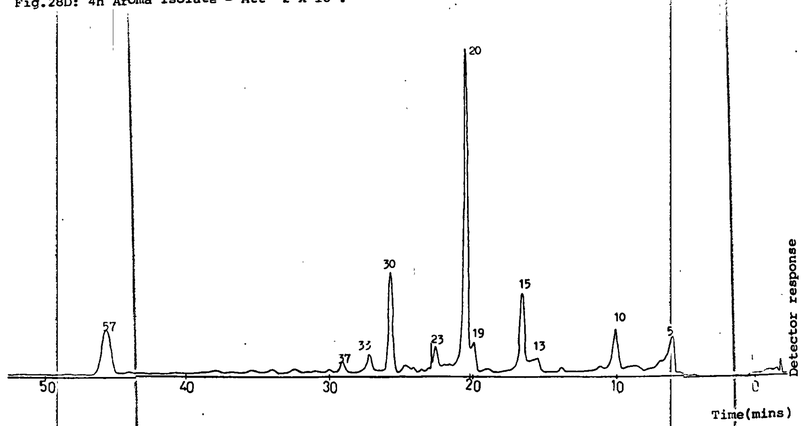


Table 12: Mass Spectra, Identities and Odour Port Descriptions of Components in 1h Aroma Isolate - Expt. 9c

Peak No.	Identity	Certainty of Identity	Observed Mass Spectra (m/e, %)	Mol wt. from CI	Ref. Spectra	Odour Port Assessment (OPA) - Expt. 15
1.	Air					
2.	Carbon dioxide	***	29 44 100 55	44	168	
3.	Pentane	***	43 41 42 29 27 39 57 100 55 53 25 24 19 9		168	
4.	Hexamethyldisiloxane	***	147 148 73 66 149 100 17 17 9 6	162	168	P-chemical solvent
5.	Heptane	***	43 41 71 57 100 82 75 60		168	
6.	Acetaldehyde	***	29 44 43 42 41 100 60 24 17 10	44	168	P-mushy,unpleasant,sulphurous faecal, H_2S, rotten eggs
7.	Trimethylethoxysilane	***	103 75 73 104 58 100 49 20 15 10		168	P-dry cleaning solvent
	2-methylpentane	***	57 41 56 43 71 39 27 100 55 53 33 24 23 16		168	V-musty P-sweet, estery,face cleanser methylated spirits,chemical solvent,ether,acetone
8.	Methanethiol	***	47 48 45 46 33 100 95 50 12 8		168	P-rotten vegetables, rotten Camembert,faecal,off, bad
9.	a hydrocarbon- poss. methylpropane	?	43 58 27 42 39 100 19 12 12 11		)))	
	acetaldehyde	***	43 29 42 44 100 60 35 34	44	168))	P-fruity, cut green leaves, fragrant
10.						*P-sickly,sweaty,rancid,faecal* rotten vegetables,meaty-boiled.
11.						P-cooked vegetables,canned sweetcorn
12.						P-chemical solvent,H_2S,burnt meaty V-faint pyridine-like,stale, green
13.						P-musty,mouldy,oily,fatty, slight cooked onion,cooked cabbage
14.						P-sweet,buttery,popcorn
15.	Acetone	***	58 43 29 42 100 89 52 27	58	168	P-chemical solvent,metallic
	Carbon disulphide	***	76 78 100 15		168	
16.						P-roast pork,baked bread crusts
17.	Dimethylsulphide	***	47 62 45 46 35 61 100 83 55 45 35 18		168	P-faint warm rubber,meaty V-buttery,slight caramel, creamy,butterscotch V-meaty,savoury,beef,gravy, pleasant
18.						P-very fishy,slightly rancid, unpleasant
19.						P-burnt, roasted, meaty,catfood meaty roast
20.						V-meaty,stale,catfood
21.					)	
22.					)))	formalin,chemical solvent, resinous,green,Stilton cheese preserved dog fish
23.	a C_4 amine,MW73	**	58 41 44 43 72 100 55 33 28 18	73	168	P-meaty roast, pleasant
24.	propanal	***	41 29 27 57 58 42 40 100 88 62 28 26 15 14	56	168	P-chemical solvent
	methyl propanal	***	43 41 58 39 27 72 29 100 28 23 20 18 15 13	72	168	
						V-meaty
25.	methanol	*	28 29 32 30 31 33 100 100 100 40 15 4		168	
26.	ethanol	***	31 28 43 30 15 100 78 25 25 23		168	
	1,1,1-trichloroethane	***	97 99 61 63 83 100 56 56 21 14		168	
27.	3-methylbutanol	***	29 58 39 41 43 100 75 63 50 29	86	168)	
28.	H_2O	***			)	P-toasted bread,meaty,sweet, diacetyl,caramel,toffee, candyfloss,buttery,butter-scotch
29.	butan-2,3-dione(diacetyl)	***	43 86 100 6	86	168)	
	trimethylsilanol	***	75 47 76 77 100 23 6 1	90	168	P-catty, fishy
30.	pentan-2-one	?	43 27 29 57 86 41 39 58 100 27 24 21 14 12 9 7		168	P-burnt,chemical solvent, pungent, buttery V-acrid,sickly,musty,mouldy, puffed wheat,wet clothes
31.						P-very very burnt,burnt fat, beefy,dripping,meaty, smoky,bacon,roast nuts
32.						P-oily,sweaty
33.	but-2-en-1-al (crotonal)	***	41 39 42 69 70 100 70 38 21 21	70	168	P-green,sap,woody,sharp, fragrant
34.	toluene	***	91 92 65 63 90 51 100 60 18 12 12 10		168	P-slightly rubbery,fruity, sickly,blue cheese,rotten cabbage
						V-burnt,faint green pepper,
35.	n-hexanal	***	44 41 28 43 57 39 72 100 85 85 65 62 38 18	100	168	P-fishy,fish paté,green beans cooked green vegetables, slightly meaty,oily,rancid fat
36.	dimethyldisulphide	***	94 45 46 47 79 48 61 100 92 78 63 53 33 22	94	168	P-stale potato crisps,burnt, smoky, rotten cabbage. V-charred
37.	butan-1-ol	***	31 41 43 56 100 33 32 16	74	168	P-stale,green vegetable, faintly meaty V-meaty boiled
38.	2,4,5-trimethyl-3-oxazoline	**	44 43 42 72 40 71 69 113 100 80 75 28 27 12 12 3	113	137	P-burnt,roasted,cereal,meaty, roast meat,dripping,pork, charred
						V-meaty boiled
39.						P-sweet,buttery,caramel,roast meat,savoury,slightly rancid oily
40.	a methylpentanolactone	*	42 41 31 55 43 57 70 39 100 93 90 72 67 33 32 32			P-estery,floral,violet,very pleasant,fruity
	hexamethylcyclotri-siloxane	***	207 233 208 209 224 96 191 133 100 37 19 14 14 14 12 9		168	P-flat,green,burnt rubber, soapy
41.	pentan-1-ol	***	42 31 41 55 29 70 100 74 56 55 43 14			P-musty,mouldy,oily,fatty, cardboard,lamb,cold meat, sharp green,rancid fat, chemical solvent, ripe Camembert
42.						P-animal,goaty
43.	chlorobenzene	***	112 77 50 51 114 100 52 10 10 29	112	168	P-buttery,oily,fatty,lamb, meaty,roast,savoury,smoky, beef,Oxo,gravy
44.	a C_7 hydrocarbon	?	57 45 71 85 100 48 24 17			P-pungent,chemical solvent, slightly rancid,buttery, nutty
45.	octamethylcyclo-tetrasiloxane	***	281 73 282 283 100 91 27 9		168	P-faint cereal,rubber,rotten vegetables,beefy
						V-coconut
						V-smoky
46.						P-estery,floral,fragrant, sweet,vanilla,cut grass, fruity
47.	3-hydroxybutanone (acetoin)	***	45 43 29 88 100 80 29 2		168	P-sweet,baked cakes,cheese
48.						P-stale,coffee,burnt,charred, nutty,hazelnuts
49.						P-burnt,charred,caramel,nougat praline,nutty,unpleasant, meaty,spicy, sausage
50.						P-vanilla,nutty,walnuts,baked cakes,pastry,buttery
51.						P-musty,mouldy
52.	hept-3-ene	**	41 29 55 56 39 98 100 85 50 47 36 8		168	P-stored fruit,fresh celery, damp newspaper,eating apple fruity,fragrant,sweet, floral,nutty,almonds
53.						P-coconut,green,floral
54.						P-sickly sweet,meaty,sweaty, charred beef,earthy
						V-charred,burnt,roast,caramel meaty,unpleasant,beef, Marmite.
55.						P-burnt milk,buttery,sweet, creamy,baked cakes,toffee-mallow
56.						P-green,wet grass,cucumber, bell-peppers V-cured meat,meaty
	dimethyldisulphide	***	45 79 47 46 12 7 64 80		168	P-smoky,chemical solvent

			100	55	33	28	18						P-meaty roast, pleasant
24.	propanal	***	41	29	27	57	58	42	40		56	168	P-chemical,rubber,acetone
	tetrasiloxane		100	91	27	9							vegetables,beefy
													V-coconut
													V-smoky
46.													P-estery,floral,fragrant, sweet,vanilla,cut grass, fruity
47.	3-hydroxybutanone (acetoin)	***	45	43	29	88						168	P-sweet,baked cakes,cheese
			100	80	29	2							
48.													P-stale,coffee,burnt,charred, nutty,hazelnuts
49.													P-burnt,charred,caramel,nougat praline,nutty,unpleasant, meaty,spicy, sausage
50.													P-vanilla,nutty,walnuts,bake cakes,pastry,buttery
51.													P-musty,mouldy
52.	**hept-3-ene**	**	41	29	55	56	39	98				168	P-stored fruit,fresh celery, damp newspaper,eating appl fruity,fragrant,sweet, floral,nutty,almonds
			100	85	50	47	36	8					
53.													P-coconut,green,floral
54.													P-sickly sweet,meaty,sweaty, charred beef,earthy
													V-charred,burnt,roast,caramel meaty,unpleasant,beef, Marmite.
55.													P-burnt milk,buttery,sweet, creamy,baked cakes,toffee-mallow
56.													P-green,wet grass,cucumber, bell-peppers
													V-cured meat,meaty
57.	*dimethyltrisulphide*	***	*45*	*79*	*47*	*46*	*12*	*7*	*64*	*80*		*168*	*P-smoky,chemical solvent, sweet,fruity,perfume, inhaler*
			100	*21*	*17*	*14*	*10*		*8*	*3*			
													V-meaty,oily,fatty,musty, mouldy,goaty,roast beef,Oxo, gravy,well-done beef,savoury
58.													P-sickly,meaty,pork,burning rubber,roast potato,savoury crisps
59.													P-charred,green pepper,roasted nuts
60.													P-Bovril,meaty,charred,smoky burnt,beef-flavour crisps, charred beef,potato crisps
													V-fruity,estery,amyl acetate cheese
													V-meaty,musty,mouldy
61.	**octan-1-ol**	**	29	41	55	56						168	P-buttery,nutty,almond
			100	70	30	32							V-cooked meat,slightly Oxo, caramel
62													P-cucumber,slight disinfectant,green pepper
63,	**benzaldehyde**	***	51	77	50	106	105	52	78		106	168	P-very strong nuts,rancid fat or batter,almonds,fragrant oily,fatty,nutty,ground almonds
			100	68	49	48	46	24	8				V-green pepper
64													P-cold roast potato,peppers
65.													

Footnote: *** indicates absolutely positive identification from good match reference spectra
** indicates very slight mis-match with reference spectra
* indicates tentative identification
? possible identification, unconfirmed.

P represents OPA of peak
V represents OPA of valley between peaks

Table 13: Mass Spectra and Identities of Aroma Components in 4h Sample - Expt. 9d.

Peak No.	Identity	Certainty of Identity	Observed Mass Spectra (m/e,%)							Mol.Wt from CI	Reference spectra
1.	carbon dioxide	***	44	28	45					44	168
			100	45	4						
2.	hexamethyldisiloxane	***	146	149	66	148				162	168
			100	18	9	9					
3.	hexane	***	57	86	68						168
			100	35	23						
4.	trimethylamine	***	58	59	42					59	168
			100	47	17						
5.	carbon dioxide	***	44	28	45	46				44	168
			100	7	4	2					
6.	carbonyl sulphide	***	60	32	61					60	168
			100	6	2						
7.	acetaldehyde	***	44	43	29	42	41	28		44	168
			100	78	33	2	12	11			
	acetone (premature elution)	***	43	58	42	28	44	39		58	168
			100	37	8	5	3	3			
8.	sulphur dioxide (premature elution)	?	64	48							
			100	54							
9.	trimethylamine	***	58	59	42	28	30			59	168
			100	43	10	4	3				
	unknown		57	56	62	86	47				
			100	44	22	17	17				
10.	unknown		74	59	45	40	89				
			100	83	18	8	6				
11.	carbon disulphide (premature elution)	***	76	78							
			100	10							
12.	dimethylsulphide	***	47	62	45	61	46			62	168
			100	82	60	28	25				
	dimethyldisulphide (premature elution)	**	48	47	45	94	79				168
			100	60	45	42	19				
	furan	*	68	39	29						168
			100	31	21						
13.	carbon disulphide	***	76	44	78	77				76	168
			100	12	10	3					
14.	ethanethiol	***	62	47	61	45					168
			100	52	33	28					
15.	propanal +	***	28	58	57	29	30			58	168
			100	75	54	35	25				
	methylpropanal	***	43	41	72	39	29			72	168
			100	20	44	12	5				
	hexamethylcyclotrisiloxane (premature elution)	***	207	208	96	209	191	133	177	222	168
			100	32	17	13	10	9	4		
	unknown		72	43	41	39	73	42	57	72	
			100	67	47	20	18	6	5		
16.	2,5-dimethylfuran	***	82	81	53	54	39			82	168
			100	58	32	8	4				
17.	unknown		72	43	41	28	39	42			
			100	85	40	18	12	6			
18.	3-methylfuran	***	82	81	53	54	39	51	52	82	168
			100	60	30	6	6	6	5		
	tetrachloromethane +	***	117	119	121	84					168
			100	95	30	20					
	unknown		43	72	57	45	60	59		72	
			100	80	26	15	15	11			
19.	2-methylbutanal	***	57	58	41	44	71	86	39	86	168
			100	100	63	44	42	33	28		
20.	3-methylbutanal	***	58	44	41	43	71	39	86	86	168
			100	95	83	62	57	39	24		
21.	an ethylfuran	***	81	96	53	44	95			96	168
22.			100	56	15	11	10				
23.	trimethylsilanol	***	75	76	45	47	77			90	168
24.	sulphur dioxide	**	64	48	66						168
			100	29	6						
25.	unknown (prob. a mixture)		91	87	69	93	37	45	73		
			100	100	70	40	28	25	22		
26.											
27.											
28.	unknown (prob. a mixture)		101	71	93	57	43	83	92		
			100	40	28	25	24	20	5		
29.	unknown (prob. a mixture)		93	71	57	92	43	32	101		
			100	100	65	25	15	15	15		
30.	dimethyldisulphide +	***	94	79	45	46	47	61	48	94	168
	a furanoid compound (prob. methylfuroate)	*	95	96	97	81	80				
			100	14	14	9	4				
31.	unknown		75	149	65	133	29	84	48		
			100	98	76	46	46	43	25		
32.	a methylthiophen	***	97	45	98	58	53	39		98	168
			100	25	20	12	7	6			
33.	2,4,5-trimethyl-3-oxazoline	**	72	43	42	44	98	113	54	113	137
			100	46	35	20	20	12	6		
34.	unknown		84	58	69	68	71	70	98		
			100	55	55	52	43	36	22		
35.	2,4-dimethyl-3-oxazoline	**	84	58	69	68	42	40	99		137
			100	52	15	14	12	9	3		
36.	ethylmethyldisulphide	***	108	80	110					108	169
			100	95	4						
37.	a methylpentanolactone	*	70	43	58	71	57	55	85		
			100	75	10	55	53	52	25		
38.	hexamethylcyclotrisiloxane	***	207	208	191	209	96	193	177	222	168
			100	22	21	15	11	6	2		
39.	a thiophen +	*	112	43	75	97	69	55	111	53 112	168
			100	53	36	35	34	34	21	5	
	unknown		107	122	77	108	39	91			
			100	54	14	14	7	7			
40.	2-n-pentylfuran	***	81	82	138	53	95			138	168
			100	24	15	9	7				
41.	chlorobenzene	***	112	77	114	51				112	168
			100	53	31	8					
42.	unknown		98	105	71	93	85	69			
			100	59	52	46	40	36			
43.	a methylpyridine+	***	93	66	92	65	67			93	168
			100	29	19	10	8				
	a C_{17} hydrocarbon	*	57	71	85						
			100	80	54						
44.	unknown		84	110	83	111	82				
			100	33	30	15	7				
45.	octamethylcyclotetrasiloxane	***	281	73	282	283	207	265	249	296	168
			100	54	30	19	18	5	3		
46.	2-methyltetrahydrofuran-3-one	*	43	72	100	44	29				168
			100	91	78	45	14				
	unknown		55	136	69	68	41	82	119	81	
			100	59	53	51	5	50	40	35	
47.	3-hydroxybutanone (acetoin)	***	45	43	88	42				88	168
			100	57	32	4					
48.	unknown		43	74	111	110					
			100	36	28	19					
49.	unknown		98	71	99	153	138				
50.			100	36	20	10	8				
51.	a dimethylpyrazine (2,5- or 2,6-)	**	108	42	81	109	40	39		108	168
52.	unknown		84	83	85						
			100	20	12						
53.	unknown		71	85	108	125	141	152	167	183	
			100	53	30	30	25	9	8	5	
54.	unknown		57	82	98	70	55	95	107		
			100	95	83	62	60	42	37		
55.	an ethylmethylpyrazine	***	121	122	94	93				122	168
			100	65	22	22					
	unknown		73	86	127	88	47	64			
			100	97	69	66	38	31			
56.	acetic acid	***	60	43	45	42	29			60	168
			100	61	40	11	2				
57.	dimethyltrisulphide	***	79	126	45	47	64	111	128	96 126	168
			100	96	24	23	20	16	13	12	
58.	a dimethylpyrazine	***	108	109	42	81	40	39			168
			100	98	42	81	40	39			
59.	a thiazole	***	85	126	71	140	99	84	141		170
			100	97	59	23	20	8	8		
60.	2-furaldehyde	***	96	95	39	40	29				
			100	89	10	9	2				
	2,4-dimethyl-5-ethylthiazole	***	126	85	141	45	59	113		141	171
			100	58	50	31	10	10			
61.	2,6-dimethyl-3-ethylpyridine	*	120	135	121	77	134	119	79	135	168
			100	54	19	5	4	3	2		
	decan-2-one	***	58	71	59	43	57	55	156	156	168
			100	50	35	28	28	13	5		
62.	a C_5 substituted pyrazine (2,3,5,6-trimethyl?)	***	149	150	39	122				150	168
			100	86	57	36					
	unknown (major component)		67	74	73	45					
			100	70	46	27					
63.	a benzenoid compound	***	91	92	119	122	162	150			168
			100	71	70	32	17	13			
	2-isopropyl-4-methyl-5-ethylthiazole	***	154	169	41	85	155			169	159
			100	30	22	16	5				
64.	2-propyl-4,5-dimethylthiazole	**	45	127	57	126	71	86	85		168
			100	27	20	14	3	3	1		
65.	benzaldehyde	***	106	105	77	51	78	50	52	106	168
66.	unknown		76	40	44	61	80	87	136		
			100	98	62	45	32	30	28		
	unknown		107	155	154	116	120	121	135	162	
			100	79	68	40	31	30	29	24	
67.	butanoic acid	***	60	73	43	45	42	41		88	168
			100	40	8	8	7	6			
69.	a C_7 substituted alkylthiazole or an acylthiazole	**	141	126	135	28	168	78	150	183	183? 159
			100	50	48	29	27	25	23	5	
70.	benzothiazole	?	135	120	98	97	70	81	108	162	114
			100	50	30	22	18	16	15	12	
71.	hexanoic acid	***	60	73	87	61	43	41			168
			100	60	36	10	7	6			
72.	3-isopentyl-2,5-dimethylpyrazine	***	122	162	71	85	127	135	121	163	172
			100	47	27	22	15	15	8	5	
76.	2-(hydroxymethyl)-furan	***	98	97	41	81					173
			100	12	10	5					
81.	dodecan-1-ol	***	70	57	83	97	111	126	125	140	174
			100	88	60	48	45	40	39	20	

Footnote: *** indicates absolutely positive identification from good match reference spectra
** indicates very slight mis-match with reference spectra
* indicates tentative identification
? possible identification unconfirmed

Fig.32A: Chromatogram of "Large Peak Fraction"
- Part III(b)

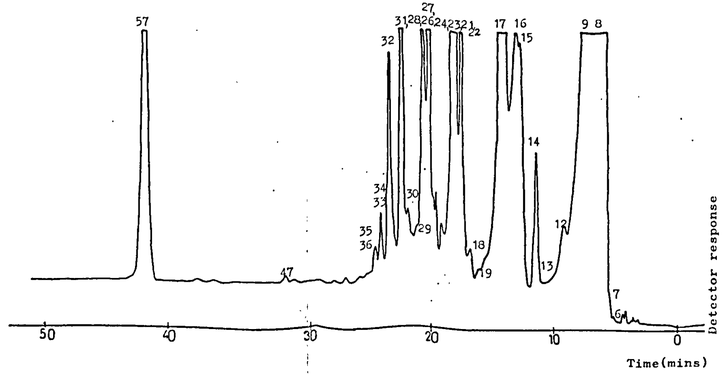


Fig.32B: Chromatogram of "Small Peak Fraction"
- Part III(b)

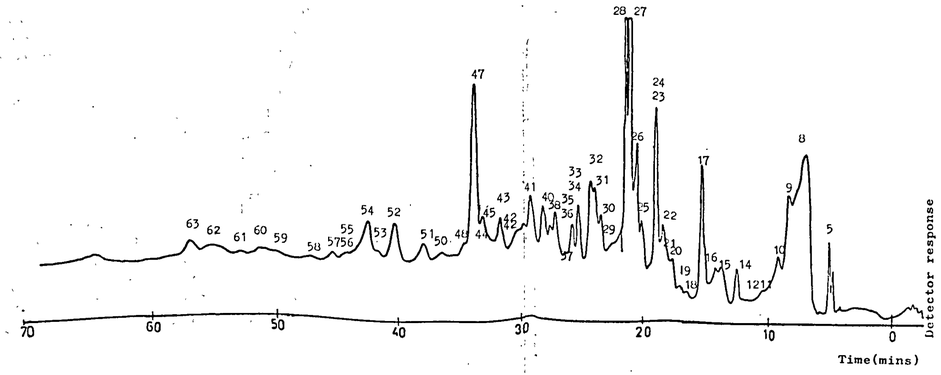


Fig.31: 1h Aroma Chromatogram Showing GC 'meaty' and 'roasted/toasted' Areas
- Part III(b)

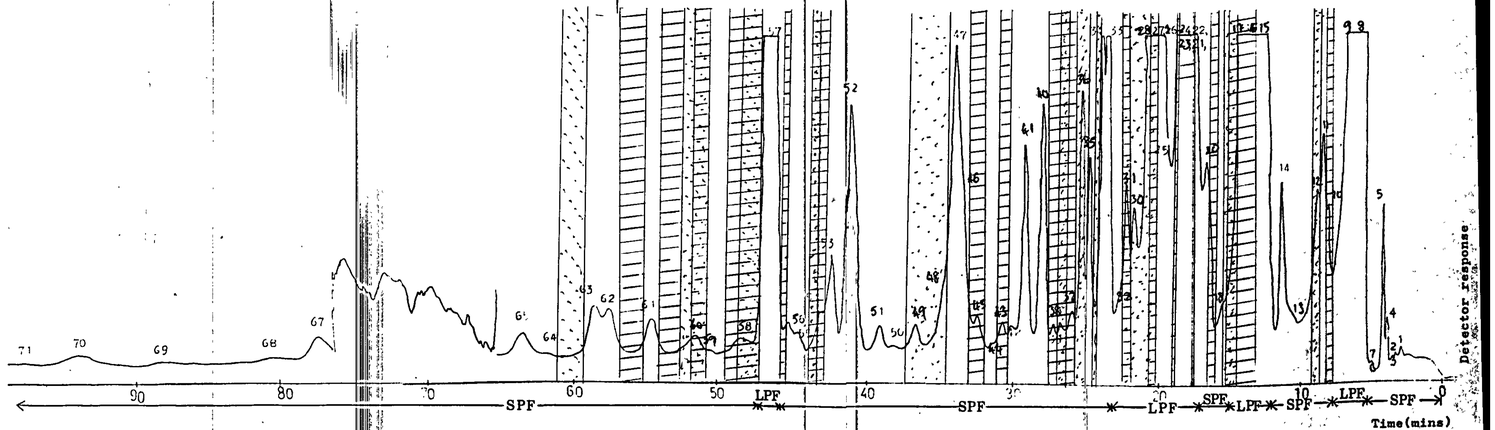
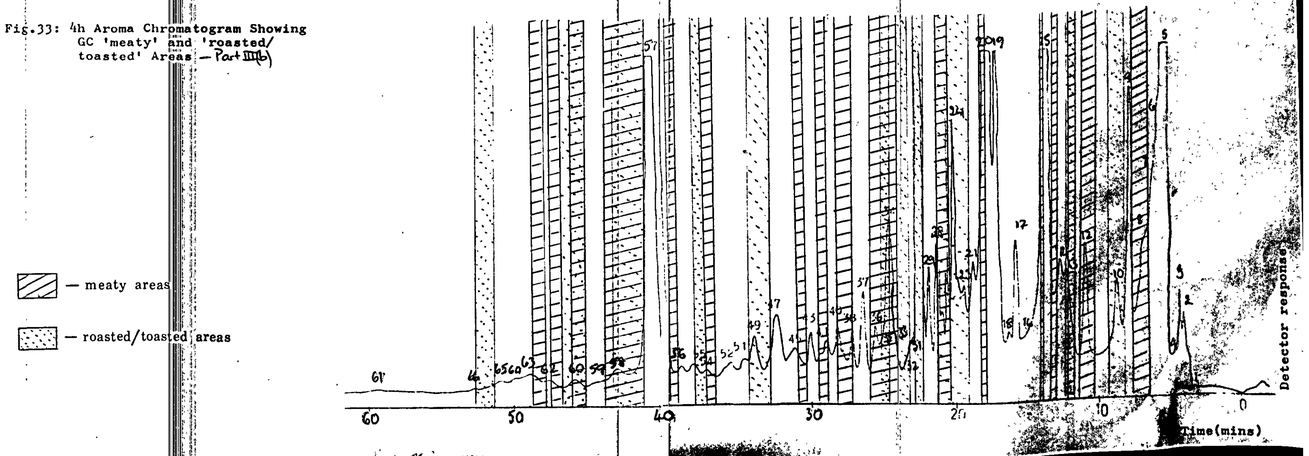


Fig.33: 4h Aroma Chromatogram Showing GC 'meaty' and 'roasted/toasted' Areas — Part III(b)



"THE CHEMICAL COMPOSITION AND SENSORY PROPERTIES OF COOKED BEEF AROMA"

Thesis submitted for the degree of

Doctor of Philosophy

in the

Faculty of Science

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by

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ABSTRACT:

The volatile components of cooked beef aroma were isolated using a modified headspace sampling technique involving a one-step trapping of the headspace vapours by adsorption on a porous polymer, Tenax GC. Rapid heat desorption was employed to transfer the volatiles directly onto a gas chromatography (GC) column for separation. Such methods have not been used previously in meat aroma research. The techniques were developed and optimised. Odour descriptions were obtained for the separated components at a GC odour port. Identification of the aroma components, by combined gas chromatography/mass spectrometry (GC/MS), was achieved by first pooling the aroma samples to increase concentrations. A total of 78 identifications were made, including 12 compounds identified for the first time in cooked beef aroma.

A factor analysis was performed on sensory data previously obtained in these laboratories. It showed that the sensory properties of cooked beef aroma can be described by 9 factors. This was validated experimentally. These odour terms were then used by untrained assessors for describing the sensory properties of different cooked beef aroma isolates desorbed from the Tenax GC using two different methods. The former involved sniffing at the exit of a short empty GC column and the latter at an odour port of a glass globe, which, by acting as a mixing chamber, enabled presentation of a 'total' aroma sample. This is novel in flavour research. These descriptive sensory analyses validated the methods used for aroma isolation, heat desorption and GC analysis.

Statistical correlation of objective and subjective data previously obtained in these laboratories was attempted by Stepwise $R_e$gression Analysis. A non-statistical correlation of the GC/MS results with the sensory results of this project indicated the importance of aldehydes, furanoids and other heterocycles (in particular 2,4-dimethyl-5-ethylthiazole) to roast beef aroma.

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Lastly, particular thanks to Mr. D.H. Moncur for without whose truly magnificent support this project would not have been completed.

DEDICATION

to the late G.C.Galt.

INDEX

INTRODUCTION

This project arose from a project also sponsored by the U.K. Meat and Livestock Commission (MLC) and previously carried out in these laboratories[1-5]. That project involved a study of the aroma components of variously heated beef, obtained by conventional cooking and heating by microwave radiation. The aromas were extracted by simultaneous steam distillation/solvent vapour extraction and concentrated by low temperature/high vacuum distillation. Individual components of the aroma isolates were separated by gas chromatography (GC) and many were identified by combined gas chromatography/mass spectrometry (GC/MS). The sensory properties of similarly prepared cooked beef aromas were described qualitatively and quantitatively by trained assessors using a vocabulary of 41 meat aroma qualities.

The aims of the present project were three-fold;
1) To obtain improved chemical data for the composition of cooked beef aroma. This work is described in Part I.
2) To rationalise and simplify the sensory qualities defined in the earlier project and to obtain further sensory data. Sensory analysis of aroma isolates (rather than cooked beef samples, as in the previous work) would provide more valid data for comparison with the equivalent chemical data. This constitutes Part II.
3) To attempt a correlation of chemical and sensory data to determine, if possible, which of the many components present contribute mostly to certain defined sensory qualities. This is described in Part III.

The project depended primarily on the success of Part I, and in particular the development of an improved and novel aroma sampling method.

PART I: THE CHEMICAL COMPOSITION OF COOKED BEEF AROMA

INTRODUCTION

Rödel and Kruse have recently defined meat flavour "as resulting from the complex sensation caused by simultaneous perception of aroma and taste substances, flavour enhancers and physical properties[6]. Of these, volatile aroma components are by far the most important.

Meat aroma is produced by chemical degradation reactions initiated in raw meat by heat, i.e. cooking. The degradative

precursors are water-soluble, low molecular weight substances which encompass 15 classes of organic compounds[7]. That these are responsible for the odours of cooked meat is easily demonstrated by thermally degrading such compounds[8,9]. The major reactions contributing to meat aroma production are:-

autoxidation, hydrolysis, dehydration, decarboxylation of lipids,

degradation of sugars, amino-acids and peptides,

complex interaction of reducing sugars and amino acids (including Strecker degradation),

reaction between hydrogen sulphide , thiols etc with other components,

degradation of thiamin,

degradation reactions of ribonucleotides[10-12].

Prior to 1960, identifications of cooked meat volatiles were exceedingly few. Improved GC and combined GC/MS techniques in the late sixties explain the rapid increase in the number of volatile compounds identified in heat-treated beef from 1965 to 1975. Since then however, a sharp drop in beef aroma volatiles identification has occurred despite relatively recent advances in sophisticated capillary GC/MS techniques. Although a large number of compounds have been identified (about 550 up to 1981[13]) it has not been possible to pinpoint the elusive chemical identities of the main contributors to the characteristic cooked beef aroma[4,14-16].

In general, aroma analysis involves three multistep stages -

(i) isolation and concentration of aroma volatiles,

(ii) separation of the individual aroma components,

(iii) identification of the individual aroma components.

A. AROMA ISOLATION AND CONCENTRATION

The two approaches to the isolation of flavour volatiles are as follows:-

(i) total volatiles analysis involving (a) distillation (flash, steam, CO_2, vacuum (fractional and high vacuum fractional), (b) solvent extraction, (c) freeze-drying, (d) derivative formation and (e) distillation of aqueous and non-aqueous extracts.

(ii) headspace analysis either direct or indirect[17].

All these methods can cause quantitative changes and some may even promote qualitative changes. It is essential to minimise artefact formation so that the isolate obtained has an aroma representative of the original food[18]. The major volatile in

many foods is water[18]; meat consists of 70% water and the application of heat will not only develop its flavour but will also produce large amounts of water vapour. The choice of method or combination of techniques should be based on the volatility of the constituents, their relative concentrations, lability, polarity and the nature of the substrate matrix[19].

(i) TOTAL VOLATILES ANALYSIS

A chronological summary of 'total volatiles analyses' applied to beef follows. Herz and Chang pumped a slurry of boiled beef under reduced pressure, and at a temperature near its boiling point, into an evaporator[20-23]. By suddenly releasing the high vacuum, a flash evaporation was effected and the residue passed as an externally-heated film down the evaporator. The volatiles were condensed in a series of cold traps. Water was separated from the trace organic volatiles during the vaporisation under reduced pressure. The collected volatiles were extracted with ether.

Tonsbeek et al. employed continuous diethylether extraction of a clear beef broth for up to 36h before concentrating by evaporation[24,25]. Weurman pointed out that direct extraction of large volumes of dilute solutions resulted in large volumes of dilute extract from which the desired aroma components had still to be isolated and concentrated[26]. During concentration, solvent impurities are accumulated and later complicate the separation and analysis of aroma components. As much concentration as possible is therefore desirable before solvent extraction. To this end, freeze concentration was recommended. However this technique has not been employed in beef flavour research.

Using previously roasted beef, and also roast beef drippings, Liebich et al. performed a 20h vacuum distillation at 55°C into two liquid N_2-cooled traps[27]. For 24h both traps were extracted with diethylether in a liquid-liquid extractor and the solvent subsequently removed by atmospheric distillation. Wilson et al.[28] and Mussinan et al.[29] performed an exhaustive atmospheric distillation of an aqueous slurry of pressure-cooked beef. After saturation of the distillate with NaCl, it was extracted in a continuous liquid-liquid extractor with diethylether. The extract was dried and concentrated by distillation.

Using the method of Flament and Ohloff[30], Flament et al. pyrolysed water-soluble extracts of raw beef and condensed the volatiles in cold traps[9,31]. The condensate was extracted with

ether and the extract separated into acid, neutral and basic fractions.

The Likens-Nickerson apparatus for simultaneous steam distillation/solvent vapour extraction of aroma volatiles, and originally devised for use with hop oils[32], was used in a modified form for the isolation of boiled and roasted beef aromas, obtained by heating conventionally and by microwaves, by MacLeod and Coppock[2,4,5]. The extracting solvent was 2-methylbutane. Low temperature/high vacuum distillation achieved concentration of the aroma isolate. The advantage of this combined technique is that extraction can be continued for as long as required. Schultz et al. tested the efficiency of 2-methylbutane and diethylether as extracting solvents and concluded that 2-methylbutane performed better for esters and aldehydes but not so well as diethylether for low boiling components[33].

The generalised decrease in beef aroma publications which occurred about 1977 was particularly related to total volatiles analyses. The next publication in 1980 used steam distillation for aroma isolation from boiled beef, followed by derivative formation of carbonyl compounds with 2,4-dinitrophenylhydrazine (2,4-DNPH) reagent[34]. A similar derivatisation technique was used to enable identification of sulphur compounds[35]. Steam distillation has several advantages including that it may be continued until all odourous materials have been exhausted, but artefact formation has been caused by this technique[36].

Shibamoto et al. also used a Likens-Nickerson apparatus for isolation of the volatile components in cooked beef (incorporated into a Japanese food called Sukiyaki)[37]. The extracting solvent was dichloromethane and concentration was achieved in an evaporative concentrator.

Disadvantages of the above methods, including the Likens and Nickerson method previously used in this laboratory, relate mainly to the use of distillation and solvent extraction techniques. The former can cause loss and changes of volatile components especially if excessive temperatures and times are involved. Solvent extraction is selective, impurities can be introduced, solvent odour is undesirable in sensory analysis and solvent removal usually involves further distillation. The long and complicated nature of several of these methods will only add to possible losses and artefact formation.

(ii) HEADSPACE ANALYSIS

Headspace gas analysis is a method of obtaining information on the contents of the volatile components of a condensed material indirectly by analysing the coexisting gas phase[38]. It is preferable for odour research to be performed by headspace analysis as the headspace actually contains the volatiles responsible for the odour in the concentration and ratios perceived by the human nose[18]. Bertuccioli stresses that the necessary integrity of the product is maintained[39].

Headspace techniques can be:

(i) direct - withdrawal of several millilitres of headspace.

(ii) indirect - concentration of headspace vapours before subsequent analyses.

Apart from its validity another advantage of direct headspace analysis is that sample handling is minimised, thereby reducing the possibility of artefact formation. However, in order to obtain sufficient concentrations of volatiles for analysis, sample injection of large headspace volumes onto a GC column is necessary and this causes poor resolution[40]. Direct headspace analysis has been used just twice for beef aroma and with little success[41,42]; thus the following survey is concerned with indirect headspace techniques.

Concentration techniques can be divided into 3, of which only the first has been extensively employed in meat aroma investigations. These are:

(a) cryogenic methods - condensation of the vapours in cold traps,

(b) absorptive methods - in a liquid,

(c) adsorptive methods - onto natural or synthetic adsorbing agents.

These remove the headspace at a rate faster than the equilibrium can be maintained and therefore cause changes in the relative concentration of the individual components. Further compositional changes may occur during trapping and recovery[43].

(a) Cryogenic Methods

Cold traps are maintained at temperatures low enough to condense aroma volatiles while permitting gases such as N_2, O_2 and CO_2 to pass through. However water will condense causing weak aroma isolates and blockage of the trap, thereby limiting the volume of headspace that may be sampled[18]. Although water has not been stated by many workers as a problem, their apparatus has incorporated various water removal components eg. drying agents, water condensers etc.

Indirect cryogenic headspace analysis of beef aroma developed rapidly from 1970 onwards. Balboni and Nawar fitted a cold trap over a flask to which a high vacuum had been applied so that the ground meat within had frozen[44]. The cold trap reservoir was filled with liquid N_2 and the sample thawed for 1h. The condensed volatiles were added to diethylether for GC analysis. Yamato et al. heated beef fat in N_2 or air and collected the volatiles in a series of reagent traps and solid CO_2/acetone-cooled traps respectively[45]. 1970 also saw the development of a method by von Sydow et al.[46], later used with canned beef[47]. This involved homogenisation of canned beef in a flask. After rotation over a 25°C water bath for 45 min., a 500 cm^3 headspace sample was passed into a cold U-trap containing 70 mesh beads.

Gas entrainment has been used widely to facilitate the transport of headspace volatiles to cold traps. Watanabe and Sato in their studies of heated beef fat and shallow fried beef employed air entrainment through cold traps before extracting the condensates with ethylether[48-51].

Brinkman et al. employed N_2 entrainment of a heated beef broth for 14 h.[52] The first cold trap was extracted with pentane and diethylether for 8 h each and the procedure repeated 20 times before analysis. Chang described a headspace method involving cooking meat gently in a jar, then bubbling N_2 continuously through the slurry and out through a series of solid CO_2-cooled traps[15]. It was aimed to decrease artefact formation in the previously described method of combined flash evaporation and vaporisation[20]. Indeed a better isolate was obtained in that the aroma closely resembled that of the original cooked meat.

Nitrogen entrainment of the volatiles of an aqueous distillate of roast beef onto a cooled glass bead-filled pipe before introduction, via a valve, to the GC was used by Mielniczuk and Daniewski[53]. More recent work using N_2 entrainment of a water slurry of roasted beef and its drippings has been carried out by Min et al.[54,55] using the method of Chang[15]. The volatiles condensed in a series of solid CO_2/acetone-cooled traps were saturated with NaCl, extracted with diethylether, dried and concentrated.

The main problem associated with cryogenic methods is the frequent need to extract the dilute condensates with organic solvent to increase concentration. The disadvantages of solvent

extraction then arise.

(b) Absorptive Methods

Indirect headspace analysis followed by absorption of the aroma volatiles in a liquid was the first headspace technique used for beef although it has rarely been used since[56]. Generally the liquids employed are derivatisation reagents specific for certain classes of compounds eg. 2,4-DNPH for carbonyls[56] and mercuric chloride and lead acetate for sulphides[57]. Other techniques for collecting the total aroma have involved collection in di-n-butylphthalate followed by solvent extraction[58] and transfer of volatiles directly to an extraction apparatus[59].

A main problem of such methods is that the volatiles require regeneration before GC analysis. This is frequently non-quantitative and can cause qualitative changes. Impurities in the derivatisation reagents and the solvents used for extraction add to the problems. Also the reagents are very dilute aqueous solutions and thus when aroma volatiles are bubbled through, loss of many volatiles occurs, especially polar components due to their solubility in water.

(c) Adsorptive Methods

Over recent years adsorptive methods have become extensively used in the analysis of trace volatiles and quite comprehensively for food aromas. This can be witnessed by the recent publication of several books devoted to the subject[60-62].

At first, natural adsorbing agents were used but since the advent of suitable synthetic porous polymers, most preconcentration of headspace vapours has been performed with the latter. Concentration depends on activity coefficients of the volatiles, which in turn depend on the presence of water, proteins, carbohydrates etc and not just on the vapour pressure or concentration of volatiles in the food[63]. The amount adsorbed depends on the initial concentration of the substance and the temperature of collection[64]. Advantages of such preconcentration techniques are that the volatiles are concentrated on the basis of their relative volatilities rather than affinity for, or solubility in, a solvent and that, compared with an isolate obtained from solvent extraction, there is a lack of solvent odour which may mask low boiling components in sensory analysis[65].

Butler and Burke found that adsorbents with the highest surface area will tend to have the highest sampling capacities[66]. However, no one adsorbent is ideal for all applications. Wyllie et al. recommended that more than one sampling technique should be used in order to obtain a complete picture of the range of volatiles present in the system[43,67,68]. Sources of artefacts as occur in "total volatiles analyses" are not applicable to entrainment of headspace vapours, although unless the entraining gases are carefully purified, then the possibility of artefact formation becomes real[69]. To this end, many purifying traps have been used to scrub the gases e.g. molecular sieves, firebrick, cold traps, P_2O_5 traps.

The volumes of headspace sampled are often quite large. Thus, problems of excess water (and ethanol in alcoholic beverages) are considerable. Several methods of removing water are commonly used, e.g. the use of hydrophobic polymers or those for which water has a short retention time. Dessicants irreversibly adsorb aroma volatiles[70]. Another solution has been to maintain the adsorbent at a raised temperature to decrease condensation[71,72].

The most widely-used method for removing water or ethanol is known as back flushing or developing. This involves flushing the polymer traps, in the reverse direction from sampling. The disadvantage is that this also removes aroma volatiles. Development time depends on the amount of adsorbent and the sampling time used[73]. Back flushing of traps has been used in the study of beverages[74-76], cigarette smoke[77] and vegetables[77,78]. The incorporation of water condensers in the sampling apparatus of several workers[80-83], can only be assumed to be intended for water removal from the headspace. Kinlin et al. used a cold water condenser and an ice trap in conjunction with the polymer trap to minimise interference by water[84]. The reflux action of the condenser also provides a stripping action as the vapours move upwards through the column. Efficiency of volatiles isolation also increases[19]. The effect of excess water vapour trapped on the polymers is to decrease gas chromatographic peak height[85]; however, unless water is condensed in the sampling trap it has no significant effect[85,86].

Having achieved adsorption of the headspace volatiles, the literature reveals an enormous variety of desorption techniques used. Basically, there are two methods - thermal or liquid.

The former has been used more extensively and has several advantages over solvent extraction, e.g. higher sampling flexibility, higher overall sensitivity and it exhibits a more homogenous behaviour to different substances[87]. Zeldes and Horton deemed backflushing during desorption to be the most critical factor[77]. Wyllie et al. compared throughflushing and backflushing and concluded that there was greater loss of volatile constituents, particularly those less volatile, when throughflushing[43]. Desorption is not always complete because of
(i) losses during transfer of traps to the analytical apparatus,
(ii) retention by chemisorption on the polymer packing,
(iii) reactions with oxygen during heat desorption, and
(iv) reactions with other sample constituents[77].

Heat desorption fundamentally involves the application of heat to the polymer tube and either a simultaneous or subsequent flow of a carrier gas to flush the volatiles from the polymer. Many different modifications of GC injection block heaters have been employed. Volatiles may be flushed
(i) into a cryogenic trap (e.g. glass U-tube[79,88,89], thin-walled glass capillary[69,90,91]) which may or may not be connected directly to the GC column,
(ii) onto a short, cooled front section of the GC column (on-column trapping[85,92,93]),
(iii) directly onto the GC column (no cooling[81,94,95]).
Recovery of the condensed volatiles in methods (i) and (ii) may involve removal of the coolant, in the presence of a carrier gas flow, and commencement of the GC temperature program for analysis. Thus the volatiles are flashed onto the GC column. Alternatively the cold trap contents may be directly injected, or added to a solvent for injection, or centrifuged and then injected onto the GC column. A survey of the properties and uses of the individual polymers commonly used for food aromas follows.

Activated Charcoal/Carbon

Several workers have used carbon to investigate volatile flavour components[96,97]. Clark and coworkers used small capillary tubes containing charcoal and heat desorbed the volatiles collected from raw walnuts directly into a high efficiency capillary column[98,99]. Recently similar methodology was used by Farley and Nursten to pass 8l purified N_2 over

malt extract[100]. Clutton and Evans tried adsorption of the flavour constituents of gin onto charcoal[101], but obtained inferior sensory results when compared with total volatiles analyses performed. Chemisorption can lead to impaired recovery of the original mixture or to the creation of artefacts[102]. Complete desorption, particularly of polar compounds, is often impossible[103].

Chromosorbs 101, 102 and 105

Table 1: Physical Characteristics of Chromosorbs 101, 102 and 105[104,105].

Chromosorb	Surface area /m^2 g^{-1}	Polymer	Temperature Limit/°C
101	< 50	styrene-divinylbenzene	275
102	300-400	styrene-divinylbenzene	250
105	600-700	polyaromatic	250

All three are recommended to be conditioned overnight to ensure stability of the polymer. Chromosorb 101 has been used for determining sulphur compounds in alcoholic beverages[95,106]. A 20ml min^{-1} N_2 stream was passed over a 20ml sample held at 20°C for 15 min. and the volatiles collected. The tube was slipped into a 200°C pressurised GC injection block to effect transferral of the volatiles to the GC column.

Using Chromosorb 102, Dravnieks et al. sampled air at 4ℓ min^{-1} through the polymer trap[107]. A modified injection port enabled flash heating of the collected volatiles onto the GC column. Early analysis was vital to prevent internal distillation which occurrs when there is a temperature gradient along the polymer-containing tube. Simpson used Chromosorb 105[108-110], in conjunction with the methodology developed by Williams and Strauss[111] in the study of the ageing of wine.

The only Chromosorb reference with respect to meat involved the use of Chromosorb 105 traps attached to the top of a Likens-Nickerson apparatus containing cooked mutton. The purpose of the trap was to collect the more volatile components escaping. Of the compounds retained, only H_2S broke through in 2-3h.

Porapaks P and Q

Table 2: Physical Characteristics of Porapaks P and Q[105].

Porapak	Surface area /m^2 g^{-1}	Polymer	Temperature Limit/°C
P	50-100	styrene-divinylbenzene-ethylvinylbenzene	250
Q	630-840	ethylvinylbenzene-divinylbenzene	250

These polymers also require conditioning prior to use to ensure stability. Jennings et al. passed N_2 via a solid CO_2/acetone trap to remove trace contaminants and bubbled it through alcoholic beverages onto a Porapak Q column which was then developed to remove water and ethanol before heating to 100°C and flushing the volatiles onto a solid CO_2-cooled trap[73]. This was centrifuged to yield 2µl essence. This method, with appropriate adaptions, has been used for investigating the volatile constituents of beer[74], cantaloupe[112] and roses[113].

The volatiles of an atmospheric steam distillation of a slurry of roasted coffee were passed over Porapak Q, then extracted with ether[114]. Porapak Q was shown by Krumperman to give erroneous peaks at temperatures > 170°C[115]. Shibamoto and Russell used Porapak Q to study the meat-like aroma generated in a model system of D-glucose-H_2S-NH_3; although the mixture had an aroma very like cooked beef, that desorbed from the polymer had a slightly different aroma[116].

Porapak Q coated with 1% PEG 20M, to decrease adsorption of high boiling components from fermented cider volatiles, was used by Williams et al.[111,117]. The N_2-entrained volatiles were then heat desorbed at 150°C and a 30ml min^{-1} flow of N_2 flushed them into a syringe. Recently the flavour components of plums has been investigated by air entrainment onto Porapak Q for 12h[89]. Heat desorption took place at 130°C[117].

The only reference to the use of Porapak Q for beef aroma described the headspace analysis of the heat-generated volatiles of beef flavour precursors by Bryant in 1970[119]. Only 11 components were identified.

Tenax GC

Tenax GC is based on 2,6-diphenyl-p-phenylene oxide and,

compared with the other polymers, has the lowest surface area (19 m^2 g^{-1}) and the highest temperature limit (375°C). Above this temperature there is a decrease in trap life[120]. It has a very high affinity for organic compounds. Conditioning and reconditioning are very important with respect to quantitation[120].

Tenax GC has been used for the analysis of many trace volatiles besides food aromas e.g. pesticides[121] and pollutants in air[87,122] and water[80,123]. Of particular note is the methodology developed by Zlatkis et al. A glass trap insert, containing the polymer, was designed to fit tightly in a modified GC injector port[82]. Helium entrainment at 20ml min^{-1} over a 100-200ml urine sample at 100°C transported the headspace vapours through a condenser to the trap insert. This was heat desorbed at 300°C for 20min. and the volatiles transferred to a solid CO_2-cooled coil inside the GC at a rate of 20ml min^{-1}. This method has subsequently been used extensively for air pollution[83,124] and body metabolites in serum and plasma[125] and urine[126,127]. Their combined findings showed that Tenax displayed a selectivity with respect to high vs. low molecular weight compounds, but that it completely retained sulphur compounds. Breakthrough from one trap to the next in series was mostly affected by sampling rate and temperature. Also it was possible to increase the total sample size by heat desorbing 2 or 3 adsorbent traps in parallel into one pre-column coil, and sample yield per experiment could be increased by positioning several traps in parallel and collecting equal amounts of volatiles on each.

Micketts and Lindsay studied beer flavour compounds using Tenax in 250mm x 6mm tapered pyrex tubes[120]. After entraining by a stream of purified N_2, the tubes were heated and back-flushed into a liquid N_2-cooled coil. The liquid N_2 was then removed and rapid resistance heating of the coil transferred the volatiles to the GC column. A comparison of Porapak Q and Tenax GC showed the latter to give better resolution for the small peaks, and desorption of high molecular weight compounds was easier because of the higher temperature limit of this polymer.

Excellent work by Novotný et al. used sage leaves for the study of some analytical aspects[85]. Breakthrough of volatiles from the polymer is affected by adsorption coefficients of

headspace constituents for Tenax and are directly responsible for concentration effects, i.e. less volatile mixtures are more effectively concentrated. The sorption equilibrium for lower boiling components is quickly reached and thus increased sampling times will only benefit higher boiling components.

More recently Zeldes and Horton found that Tenax of mesh size 35/60 was the better adsorbent(compared with Tenax 60/80) because of its higher total pore area[77]. They also concluded that decreasing the internal diameter of the sampling trap led to better resolution and an increase in GC retention time. This is supported by Williams et al.[128].

Noble and coworkers displaced a wine sample headspace onto Tenax traps by adding the same wine at a rate of 20ml min^{-1} [75,129]. After development the volatiles were heat desorbed at 130°C and N_2-flushed into cooled spiral traps for 25 min. Flash heating transferred them to the GC column. A slight variation involving displacement of the headspace by the piston movement of a syringe has more recently been used[76].

Palkert and coworkers very recently flushed N_2 through a sample of flavoured, textured soy protein onto 0.6g Tenax at a rate of 25ml min^{-1} for 30 min[88,130]. Heat desorption using 13ml min^{-1} N_2 for 15 min. at 225°C transferred the collected volatiles to a liquid N_2-cooled capillary column and from there onto the GC column. Buckholtz et al. used 40mg Tenax and flushed N_2 through a column containing peanuts at 50°C and then onto 3 traps in series for 4h at 40ml min^{-1}. The volatiles were heat desorbed onto a glass capillary GC column[90]. Possibly the most interesting feature of the method developed by Tsugita et al. to study soybean volatiles is the heat desorption system[131]. Volatiles collected by N_2 entrainment onto 200mg Tenax were heated to 200°C by a heater coiled around the polymer tube. When N_2 was diverted through the tube the volatiles were flushed via a stainless steel needle directly onto a capillary GC column in 20 seconds. After 'injection', N_2 was rediverted to by-pass the Tenax tube, and GC analysis commenced.

Adsorbents have been compared as follows. Withycombe et al. assessed Porapak Q to be more efficient than Tenax, based on the number of compounds identified, for the volatile constituents of hydrolysed vegetable protein[79]. Several workers have

concluded that Porapak Q is better for trapping low boiling compounds and Tenax for those with higher boiling points[67]. Boyko et al. compared corn odours trapped on Tenax, Porapak Q and Chromosorb 102 and pointed out that a polymer capable of trapping higher boiling volatiles is important if these volatiles are of particular interest[71]. Simon et al. analysed carrot volatiles by a distillation method and by N_2 entrainment of the volatiles onto Tenax followed by elution with ethylether[78]. The accuracy of carrot volatile collection on Tenax was greater than that with distillation for nearly all the compounds.

In their comparisons of a range of Chromosorbs and Tenax GC, Barnes et al. showed recently that Chromosorb 103 provided the highest recoveries for a model system of some typical food aroma components, if solvent elution (using acetone) was used for desorption[103]. This would therefore be the method of choice for aromas not produced thermally. Sampling efficiency increased with sampling time, the optimum being 30 min. Sampling flowrate was not so critical but approximately 200ml min^{-1} was appropriate. However, when heat desorption was used, Tenax GC (desorbed at 250°C) gave the highest recoveries; in fact almost quantitative desorption occurred. In this case sampling efficiency decreased with sampling time (breakthrough presumably occurring during sampling) and therefore a higher sampling flowrate for a shorter time was more efficient. Heat induced aromas e.g. meat aromas are therefore more appropriately analysed this way.

Clearly, adsorption onto porous polymers - especially Tenax GC - offers many advantages.

Choice of Methods Used in This Project.

The criteria for selection of an isolation method required that it fulfilled the following:-

(i) provided mild treatment of the beef throughout cooking and isolation,

(ii) that the method be as simple as possible and as close to that normally encountered in the home ie. normal cooking period and short sampling time,

(iii) minimised artefact formation,

(iv) achieved an aroma isolate representative of the original sample,

(v) achieved aroma isolates amenable to direct sensory analysis (ie. preferably no solvent).

Thus it was decided to select one of the most direct methods which is a simple modification of headspace sampling involving preconcentration of headspace vapours by adsorption. The relative concentration and ratios of the constituents present in the headspace provide the characteristic aroma of that food and therefore headspace analysis is preferable to total volatiles analysis. During adsorption onto porous polymers, volatiles are concentrated on the basis of their relative volatilities rather than solubilities in a solvent. In a recent review, Adda and Eriksson recommended the use of mild isolation treatments such as headspace entrainment over the more harsh treatments involved in distillation and extraction techniques[132].

Because of its superior properties, including the unusual ability to reversibly adsorb organic volatiles, Tenax GC was selected as adsorbent. It has been suggested that higher boiling volatiles have collectively more meat aroma than lower boiling compounds[133]. The selectivity of Tenax to higher boiling components makes it the appropriate choice for this study. Also, with sensory analysis in view, comparisons have established that Tenax gives superior sensory results to other polymers[90].

It was aimed to capture and concentrate the aroma quickly in one stage; direct heat desorption onto the GC column was also desirable. The in-parallel concept of Zlatkis et al. (see p. 18) was particularly attractive in view of the high cost of beef.

Although indirect headspace analysis of meat volatiles has been widely performed, preconcentration has almost exclusively been by cold condensation. Although adsorptive methods have been used twice for beef volatiles, in neither case was Tenax the selected polymer. This project therefore represents a novel approach to the analysis of beef flavour volatiles.

B. SEPARATION OF AROMA VOLATILES.

Separation techniques are almost exclusively chromatographic i.e. GC or high pressure liquid chromatography (HPLC), the former being much more widely used, due to its versatility and ability to separate trace quantities of complex volatile mixtures[134]. Basically there are only two main types of GC columns i.e. packed and open tubular (capillary dimensions)[105].

Choice of Methods Used in This Project.

Glass packed columns were selected so that sample capacity could be maximised. The small sample capacities of open tubular columns have precluded the use of GC fractionation techniques and sensory analysis of the GC effluent[135]. Selection of the polar stationary phase, PEG 20M was determined by results of work previously carried out in these laboratories[2,4,5]. It gave infinitely superior resolution to the non-polar OV-101. It has also been widely used in beef aroma research (see Table 3). The flame ionisation detector (FID) is a very sensitive detector with a wide linear range of response. It is comparatively inert to water vapour which was a distinct advantage for this project.

Table 3: Gas Chromatography Conditions and Identification Methods used by Beef Aroma Researchers since 1970

Reference	Column	Stationary Phase	Detector	Identification
22	1.85m x 0.3cm packed s.s. 3m x 0.3cm packed, s.s. 6m x 0.3cm packed, s.s.	PEG 20M SE-30 OV-101	FID,TCD	MS, t_r
23	6m x 0.3cm packed, s.s. 3m x 0.3cm packed, Al	PEG 20M PEG 20M,SE-30	FID	MS, IR
24,25,136	2m x 0.4cm packed 3m x 0.4cm packed	PEG 20M Apiezon-L + PEG 20M	FID	MS, IR, UV, NMR, synthesis
27	capill, s.s.	Dowfax 9N15	FID	MS
28,29,137	capill, s.s. 3.7m x 0.95cm packed s.s.	SF-96, PEG 20M SE-52	FID,FPD	MS, t_r
9,30,31	capill, glass	XF-1105	FID	MS, NMR, synthesis
2,4,5	5.5m x 0.6cm packed glass	PEG 20M	FID	MS, t_r
3	2.7m x 0.4cm packed glass	Apiezon-M,PEG 1000 Tritone X-305		MS, t_r
35,57	2.1m x 0.4cm packed	PEG 1000, Triton X-305 OV-17, Apiezon-M	FID,FPD	t_r
138	6m x 0.25cm packed glass	PEG 20M	FID	MS, t_r
37	capill	PEG 20M, OV-101	FID, FTD	MS, t_r
45	2m x 0.3cm packed	Silicone KF-54	FID	MS,IR, NMR
47	capill, s.s. capill, s.s. 6.3m x 0.37cm packed	UCON50HB2000 SF-96+IgepalCO-880 Igepal CA-630	FID,FPD	MS
48-51	2m x 0.3cm packed 2m x 0.3cm packed	PEG 20M SE-30	FID	MS
52	5m x 0.3cm packed	PEG 20M	FID	MS,IR, NMR
15,21, 139	6m x 0.3cm packed, s.s. 3m x 0.3cm packed, s.s.	PEG 20M OV-101	FID	MS
54,55	3.7m x 0.3cm packed, s.s. 3m x 0.3cm packed, s.s. 3m x 0.3cm packed, s.s.	SE-30 OV-101 PEG 20M	TCD	MS

Key:
- s.s. = stainless steel
- FID = flame ionisation detector
- FPD = flame photometric detector
- TCD = thermal conductivity detector
- FTD = flame thermionic detector
- MS = mass spectrometry
- NMR = nuclear magnetic resonance spectroscopy
- IR = infrared spectrophotometry
- UV = ultraviolet spectrophotometry
- t_r = retention times

C. IDENTIFICATION OF AROMA VOLATILES.

It is clear from Table 3 that combined GC/MS is a widely-used analytical technique for the identification of aroma volatiles. GC/MS gives positive identifications although these are often confirmed by other techniques eg. IR, NMR, t_r (see Table 3). An advantage of MS is that it is of comparable sensitivity to that of GC. Whereas the GC detection limit (for FID) is 10^{-9}g, that of MS (for total ion current) occurs at 10^{-8}g[140]. Other physical identification methods have higher detection limits and therefore have limited applications[135].

There are two main types of mass spectral analysis:-

(i) Electron Ionisation (EI) - under high vacuum, high-energy electron bombardment causes ionisation of the sample thereby producing extensive fragmentation of molecules. The resultant positive ions are separated to give a characteristic spectrum of the molecule.

(ii) Chemical Ionisation (CI) - collision of the sample vapour with low velocity charged reagent gas molecules (i.e. not electrons) results in ionisation. The charged species are usually generated by electron bombardment of a reagent gas eg. methane. Under high pressure, frequent low energy collisions, usually involving transfer of a proton, occur without causing massive fragmentation of the sample molecular ions. Intense (M + 1) fragments give good evidence of molecular weight. When EI and CI are performed in parallel their combined information provides a particularly powerful identification tool.

More than 550 aroma compounds have been identified in cooked beef. These are listed in a recent review[13]. They include:-

63 aliphatic hydrocarbons (saturated and unsaturated)[5,15,23,47-49,55,139,141,142].

4 alicyclic hydrocarbons[15,139]

7 terpenes[15,47,55,139]

34 aliphatic alcohols (saturated and unsaturated)[5,15,23,24,27,47,49,51,55,139,143-146]

43 aliphatic aldehydes (saturated and unsaturated)[5,8,13,23,24,27,45,47,49,52,55,139,142,8]

33 aliphatic ketones[5,8,15,23,24,27,41,45,47,49,55,139,142,144-152]

6 alicyclic ketones[152,153]

20 aliphatic carboxylic acids[8,15,23,24,27,139,146,150,154,155]

32 lactones (γ and δ)[14,23,27,49,55,139,144,152,3,156,8]

10 aliphatic esters[15,21,23,41,47,49,55,139,144,151,153]

3 aliphatic ethers[15,21,23,139]

2 aliphatic amines[8,147,150]

6 chlorine-containing compounds[5,15,139,144]

67 benzenoid compounds[15,23,24,27,49-51,54,139,144]

61 non-cyclic sulphur compounds (thiols and sulphides)[5,15,21,23,24,27,41,47,50,52,55,139,143,146,147,149,150]

33 furans and derivatives[15,21,23,24,27,47,51,52,55,57,139,144,151,154,156]

35 thiophens and derivatives[5,14,15,23,28,29,47,55,57,139,156,158]

12 pyrroles and derivatives[4,5,14,15,27,29,51,139,144,153]

10 pyridines and derivatives[4,5,14,15,50,139]

49 pyrazines and derivatives including 7 pyrrolo-pyrazines and 7 cyclopentapyrazines[5,9,14,15,29-31,49,50,133,139,153,159,160]

7 oxazoles and oxazolines[14,15,23,27,137,139,148,153]

15 thiazoles and thiazolines[14,15,24,27-29,51,137,139,153,161]

12 other sulphur compounds[15,23,28,29,47,52,57,137,139,148,153,156]

2 miscellaneous compounds[14,28,52,137,153,156]

Other compounds not listed in this review are as follows:-

2 methylundecane[138]

tetradec-2,4-diene[138]

2-methylhexadecane[138]

2-methylheptadecane[138]

2-methylpentanal[34]

3-methylpentanal[34]

pent-2-enal[34]

12-methyltridecanal[138]

13-methyltetradecanal[138]

14-methylpentadecanal[138]

15-methylhexadecanal[138]

anteisoheptadecanal[138]

16-methylheptadecanal[138]

a 14-methylpentadecanal[138]

a hexadecanal[138]

a heptadecanal[138]

an octadecanal[138]

5-methyl-2-phenyl hex-2-enal[138]

2-phenyl but-2-enal[138]

nonan-5-one[34]

2-methylpentan-3-one[34]

3-methylpentan-2-one[34]

furfuryl methyl sulphide[138]

3,4-dimethylthiophen[138]

1-furfurylpyrrole[138]

2-formyl-N-(2'-furfuryl) pyrrole[138]

2,3,5-trimethylpyridine[138]

4-vinylpyridine[138]

5,7-dimethyl-6,7-dihydro-5H-cyclopentapyrazine[138]

2-ethyl-6,7-dihydro-5H-cyclopentapyrazine[138]

4-butyl-5-propylthiazole[138]

ionol[138]

3-amino-1,4-dimethyl-5H-pyrido(4,3-b)-indole[162]

3-hydroxypentan-2-one[138]
2,4-dimethylpentan-3-one
trans-pent-3-en-2-one[138]
4-methylpent-3-en-2-one[138]
5-methylhex-3-en-2-one[138]
nona-3,5-dien-2-one[138]
propiophenone[138]
2-hydroxy-3-methylcyclopent-2-en-1-one[138]
methyl (2-methyl)pentanoate[138]
methyl (2-methyl)pent-2-enoate[138]
propyl deca-trans-2,cis-4-dienoate[138]
methylbenzoate[138]
a (2-methylpropyl)-1-methylbenzene[138]
4-mercapto-5-methyltetrahydrofuran-3-one[138]

Choice of Methods used in this Project

Combined GC/MS involving EI and CI was chosen (EI only had been used for the earlier MLC project).

EXPERIMENTAL

A. AROMA ISOLATION AND CONCENTRATION

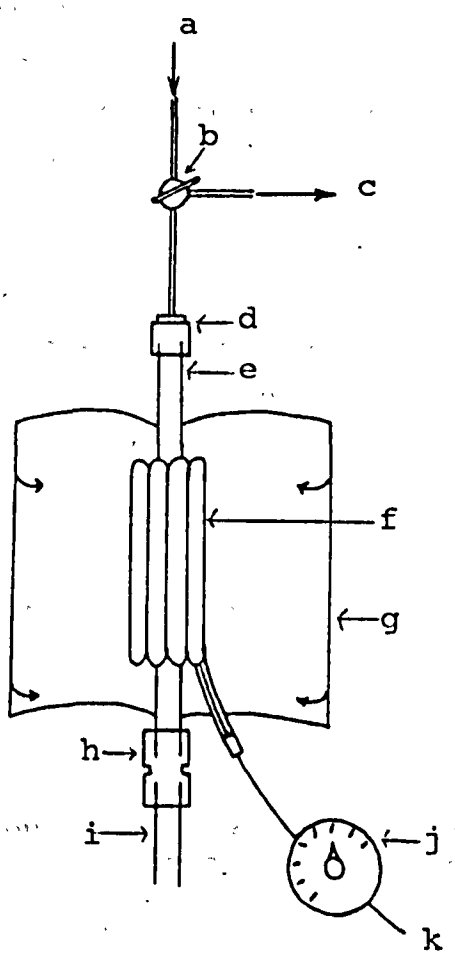
EXPERIMENT 1: Development and Preliminary Trials of the Sampling Method.

1a: Preparation of Tenax Tubes and Blank Tenax Chromatograms.

Tenax GC (60/80 mesh, Field Instruments Co. Ltd.) was conditioned by heating about 20g at 275°C/24h in a dry N_2 flow of 50ml min^{-1}. A 3cm length of Tenax GC (0.0458 ± 0.0005g), plugged with glass wool was packed in 20cm long glass tubes (6mm o.d., 4mm i.d.). A blank Tenax tube gas chromatogram was obtained by heat-desorbing the Tenax into a GC column, using the GC conditions shown in Table 4, and the following heat desorption process:

A Pye Unicam Series 104 gas chromatograph was modified by removing the normal sample injection system and replacing it with the heat desorption system shown in Fig. 1, incorporated into the carrier gas line by means of Swagelok couplings. Air was removed from the Tenax tube (thereby preventing Tenax decomposition on later heating) by passing through it a N_2 carrier gas flow of 30ml min^{-1}/10 min at room temperature. This flow was passed onto the column, rather than to atmosphere,

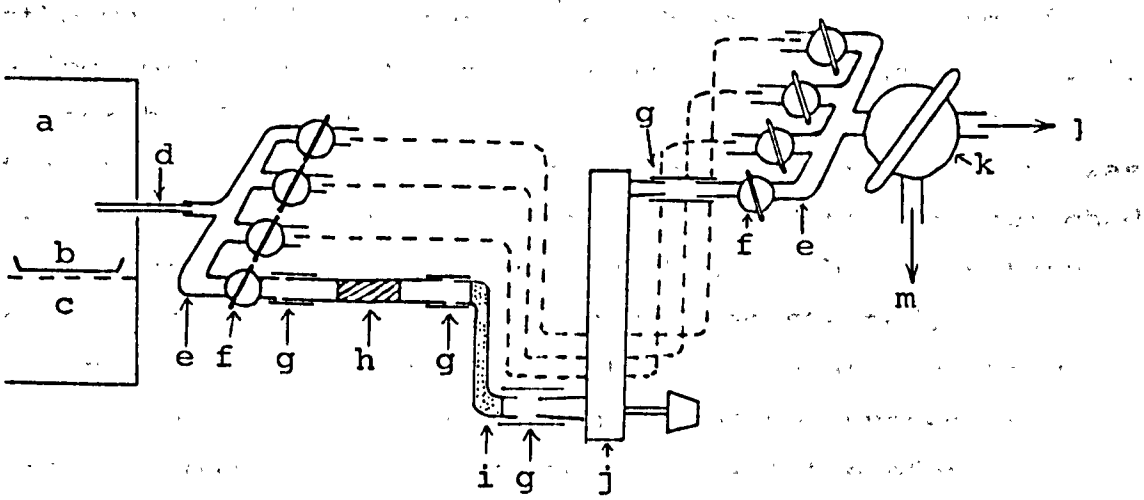
Fig. 1: Heat Desorption System.



Key:

a. N_2 carrier flow
b. 3/way valve
c. to atmosphere
d. reducing union($\frac{1}{16}$"/$\frac{1}{4}$")
e. Tenax tube
f. Heater
g. Insulating material(3"o.d.)
h. Union($\frac{1}{4}$"/$\frac{1}{4}$")
i. GC column
j. Energy regulator
k. Mains

Fig. 2: Initial Sampling Apparatus.



Key:

a. Oven
b. Beef
c. Oven dish
d. Glass tube
e. Glass manifold
f. Glass vacuum tap
g. Polythene tubing
h. Tenax tube
i. Silica gel drying tube
j. Flowmeter
k. 3/way valve
l. to vacuum pump
m. to atmosphere

Table 4: Gas Chromatographic Conditions for Samples Heat-Desorbed from Tenax Tubes.

Instrument	Pye Unicam 104 Model 64
Detector	flame ionisation
Detector oven temperature	200°C
Carrier gas	oxygen-free nitrogen (45-50 psi)
Air	hydrocarbon, moisture and carbon dioxide-free (12 psi)
Hydrogen	high purity (15psi)
Flowrates - carrier gas	30ml min^{-1}
hydrogen	30ml min^{-1}
air	500ml min^{-1}
Column:	5.5m x 6mm o.d. glass, packed with 20% PEG 20M on acid-washed Celite, 100/120 mesh
Temperature Program:	70°C for 9 min., temperature increased at 10°C/min to 150°C and held until all peaks emerged
Attenuation:	$5 \times 10^{2} = 5 \times 10^{-10}$A full scale deflection (FSD)*

* Attenuation 5×10^{2} was the most sensitive attenuation possible for achieving a satisfactory Blank Tenax chromatogram. Additionally, detectable peaks at this attenuation were necessary for subsequent mass spectral analysis.

since preliminary experiments showed that some volatiles were lost when sampled Tenax tubes were desorbed using the latter method. This N_2 flow was then vented to atmosphere during heating of the Tenax to 250°C, using an energy regulator (Electrothermal 220/240v) attached to a heater consisting of 0.6m heating cord (Electrothermal Series HC) as shown in Fig. 1. The regulator had been previously calibrated such that the temperature of 250°C was reached in 1 min. This temperature was maintained for 1 min., after which time, the heater was switched off, the carrier gas flow was diverted through the Tenax tube and GC column, and the GC temperature program started.

Additional cleaning of the Tenax tubes by heat desorbing twice into the atmosphere, considerably improved subsequent blank Tenax chromatograms. This procedure was therefore standardised in future experiments.

1b: The Initial Sampling System

The apparatus was set up as shown in Fig. 2. The vacuum stopcocks enabled the individual "in-parallel" Tenax tubes to

be switched into or out of the system. The vacuum pump enabled the oven headspace to be drawn through the Tenax tubes and flowmeters (Brooks Instruments Ltd.). Beef topside (1Kg) from a local supplier and trimmed of all extramuscular fat, was cooked in the oven at 205°C (400°F) for 60 min. During cooking, the sampling apparatus was not attached to the oven. Sampling then began using a flow of 150ml min^{-1} through each Tenax tube and sampling for different periods in each of the four tubes, i.e. sampling times of 5, 15, 30 and 60 min. respectively. The sampled tubes were heat desorbed and chromatographed as described in Expt. 1a.

1c: Blank Oven Experiment

The validity of Expt. 1b was checked by performing a blank oven experiment in which no meat was present. Other experimental conditions were as for Expt. 1a. The experiment was repeated after having thoroughly cleaned the oven and also whilst leading the vacuum pump exhaust pipe outside the laboratory.

1d: Optimisation of Sampling Flowrate and Sampling Time

This experiment was performed in an attempt to obtain stronger chromatograms, having shown in preliminary experiments that increasing the surface area of the beef did not achieve this.

i) Roast beef aroma volatiles were sampled for four different periods of time at each of four different flowrates:

Sampling flowrates: 100, 125, 175, 250 ml min^{-1}

Sampling times: 5, 15, 30, 60 min.

Other experimental conditions were as in Expt. 1b.

ii) Using two Tenax tubes in series instead of the four-tube parallel system described hitherto, roast beef aroma volatiles were sampled at 500ml min^{-1} for 15 min. The "in-series" system was designed to test for breakthrough of volatiles from the first Tenax tube.

EXPERIMENT 2: Optimisation of the Sampling Method.

2a: Attempts to Obtain Stronger Chromatograms.

Evidence obtained from Expt. 1d pointed to the use of a smaller container inside the oven to confine volatiles before sampling. Also a sampling flow > 250ml min^{-1} for a sampling time < 15 min. appeared desirable.

i) The beef was cooked in a 2ℓ Pyrex flask fitted with a

1.5cm diameter vent line positioned to ensure sampling of the entire cooked headspace. Using the apparatus shown in Fig. 3A, sampling took place at 500ml min^{-1} for 5 min. The subambient (Sba) tube, in series with the ambient (Amb) Tenax tube to minimise breakthrough, was equilibrated to -78°C in the coolant (solid CO_2/acetone) for 30 min. before sampling began. All other experimental conditions were as for Expt. 1b.

ii) The above experiment was repeated after having modified the section of apparatus in between the Amb and Sba traps as shown in Fig. 3B. An anhydrous K_2CO_3 plug was also inserted in the Y-piece (l); this, and the flask (m) were designed to prevent water from the cooked beef headspace reaching the Sba trap causing blockage during sampling. The coolant mixture was added after the whole apparatus had been set up, to minimise acetone contamination of the Tenax tubes.

2b: Experiments to Limit the Amount of Water Being Sampled.

Evidence from results of Expt. 2a indicated that further attempts were necessary to remove all or some of the water responsible for chromatographic background drift. However, this had to be achieved without prejudicing other aroma volatiles. An experiment was performed using the apparatus shown in Fig. 3C, p.30 and sampling at 500ml min^{-1} for 5 min.

A 33cm Liebig condenser was positioned vertically before the two vertical Tenax traps. A glass T-junction enabled a $50cm^3$ flask to be attached at the base of the condenser to collect the refluxed water. Additionally a needle valve was used to enable the vacuum to be introduced into the sampling system gradually. A 100ml min^{-1} flow of dry N_2 (silica gel drying agent) was passed through the Tenax tubes during the 30 min. sub-ambient temperature equilibration time. Acetone contamination of the Tenax tubes was minimised as described in Expt. 2a(ii). All other experimental conditions were as in Expt. 1b.

Also before sampling, a 100ml min^{-1} flow of dry N_2 was passed through the Sba trap (and the Amb Tenax tube for practical convenience) for the 30 min. temperature equilibration time - to flush out the air plus water vapour in it, thereby preventing blockage on cooling.

Fig.3: Diagrams of Apparatus Used in Expts.2a - e.

Fig.3A.

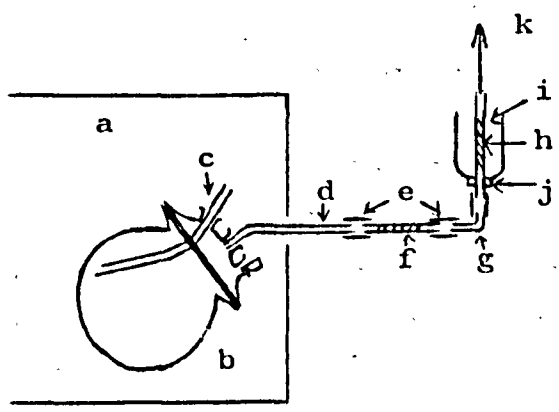


Fig.3B.

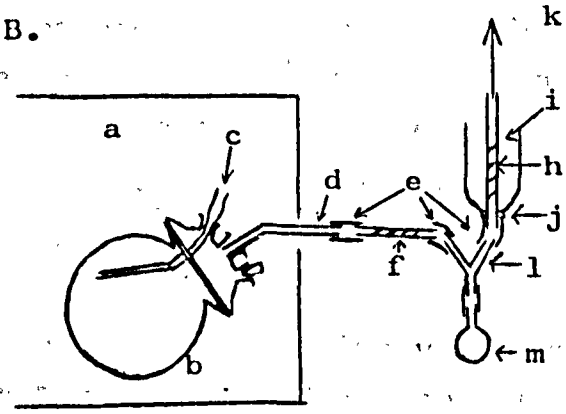
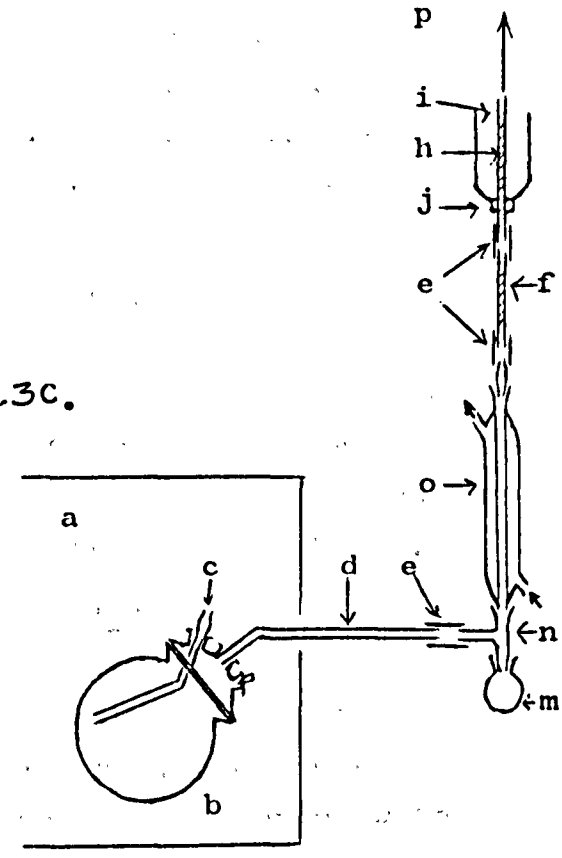


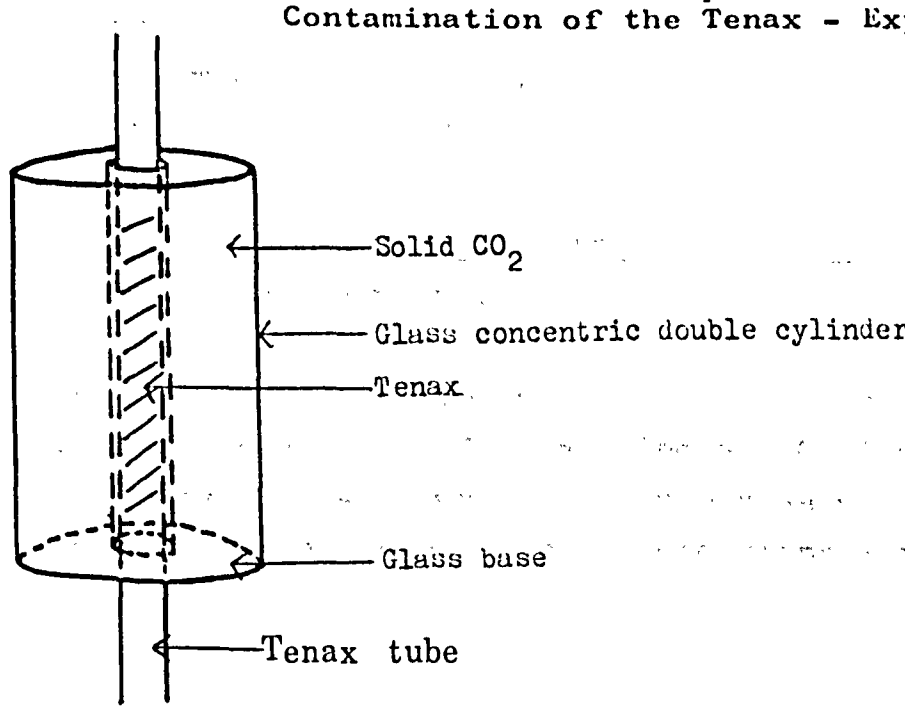
Fig.3C.



Key:

a. Oven
b. Flask
c. 1.5cm vent line
d. Glass tube
e. Polythene tubing
f. Ambient Tenax tube
g. Right-angled glass bend
h. Sub-ambient Tenax tube
i. Coolant
j. Plastic screw cap(QC/7 on SQ13)
k. 3/way valve and vacuum pump
l. Glass Y-piece
m. 50cm^3 glass flask
n. Glass T-piece
o. 33cm Liebig condenser
p. to needle valve, 3/way valve and vacuum pump

Fig.3D: Design of Efficient Coolant Trap to Prevent Acetone Contamination of the Tenax - Expt.2c.



2c: Experiment to Prevent Coolant Contamination.

Attempts to minimise acetone contamination were not always effective nor were the results reproducible. A new coolant trap was designed (see Fig. 3D, p.30) and solid CO_2 alone was used as coolant. The Tenax temperature, monitored by a thermocouple and pen-recorder, reached -76.5°C in 10 min.

An experiment was performed as in Expt. 2b but using the new design and equilibrated coolant trap. Also, Swagelok unions were used to connect the Tenax tubes together, thereby replacing the previously used polythene tubing. The GC N_2 flush of the sample tube just prior to heat desorption was reduced from 10 min. (see p.25) to 3.5 min. to minimise breakthrough before heating began. The final GC temperature was also raised from 150°C to 175°C in order to resolve more high boiling compounds. All other conditions were as in Expt. 1b.

2d: Experiment to Determine the Efficiency of the Adsorbent Traps.

An experiment was designed to determine the efficiency of the two Tenax traps used hitherto in collecting the headspace volatiles both qualitatively and quantitatively. Two sub-ambient Tenax tubes (Sba 1 and Sba 2) were used in series with an Amb tube and an experiment performed as in Expt. 2c.

2e: Experiment to Determine Optimum Sampling Flowrate and Sampling Time.

Breakthrough of volatiles from Sba 1 Tenax trap was evident from the results of Expt. 2d. Major constructional changes in the sampling apparatus since Expt. 1d, made it necessary to re-evaluate the optimum flowrate in conjunction with the sampling time.

Experiments were performed as in Expt. 2d. The following sampling combinations were assessed:

(i) 500ml min^{-1} for 10 min.
(ii) 250ml min^{-1} for 5 min.
(iii) 250ml min^{-1} for 10 min.

2f: Experiment to Determine the Minimum Number of "in series" Adsorbent Traps for Sampling.

An experiment was performed as in Expt. 2e, having omitted the Amb Tenax tube. Two sub-ambient tubes, Sba 1 and Sba 2, in series, were therefore used for sampling at 500ml min^{-1} for 5 min. This system is known as the 'one x sampling' system (1 x S system). All other experimental conditions were as for Expt. 2c.

2g: Experiment to Increase the Number of Samples from Each Beef Experiment.

1kg. beef was cooked as in Expts. 2a ——2f, after which the aroma volatiles were sampled using the apparatus shown in Fig. 4. Four in-parallel sets of two Sba Tenax traps were used in series. Sampling took place at 500ml min^{-1} for 5 min. and all other experimental conditions were as in Expt. 2c.

The large, 42cm spiral condenser (i) was necessary to deal with the increased flow at this point in this system - hereinafter known as the "four x sampling" system (4 x S system) - see Fig. 4, p.33.

2h: Evaluation of Tenax Capacity

This experiment was designed to assess whether the break-through encountered previously had been due, or in part due, to Tenax saturation.

An experiment was performed, as in Expt. 2g in which the Sba Tenax tubes contained twice the amount of Tenax hitherto used i.e. 0.09g. All other experimental conditions were as in Expt. 2c.

EXPERIMENT 3: Blank Experiment.

3a: Initial Blank Oven Experiments.

Excessive background drift appeared suddenly and inexplicably on chromatograms obtained under the conditions of Expt. 2h. Exhaustive checks on the sampling system and GC culminated in a blank oven experiment.

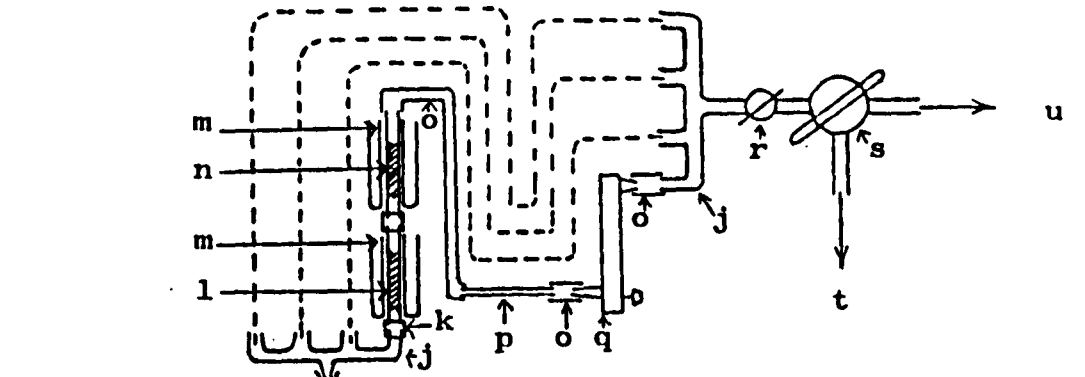
(i) Using the 4XS sampling apparatus shown in Fig. 4 (p.33) an experiment was performed as in Expt. 2h, except that the four Sba2tubes were omitted (on the evidence obtained in Expt.2h - see page 54); also the flask contained no beef. All other experimental conditions were as in Expt. 2c.

(ii) The above experiment was repeated after cleaning the oven with a commercial scouring powder, rinsing with water, then acetone and successively heating and cooling the oven in a flow of N_2.

3b: Experiments to Determine the Efficiency of the Water Condenser.

The efficacy of the condenser for the removal of cooked headspace water was investigated. Three blank oven experiments were performed as in Expt. 3a (i), using water at 8°, 12° and 14.5°C (ambient) respectively, circulating through the condenser at 1.2ℓ min^{-1} by a circulating pump. All other experimental

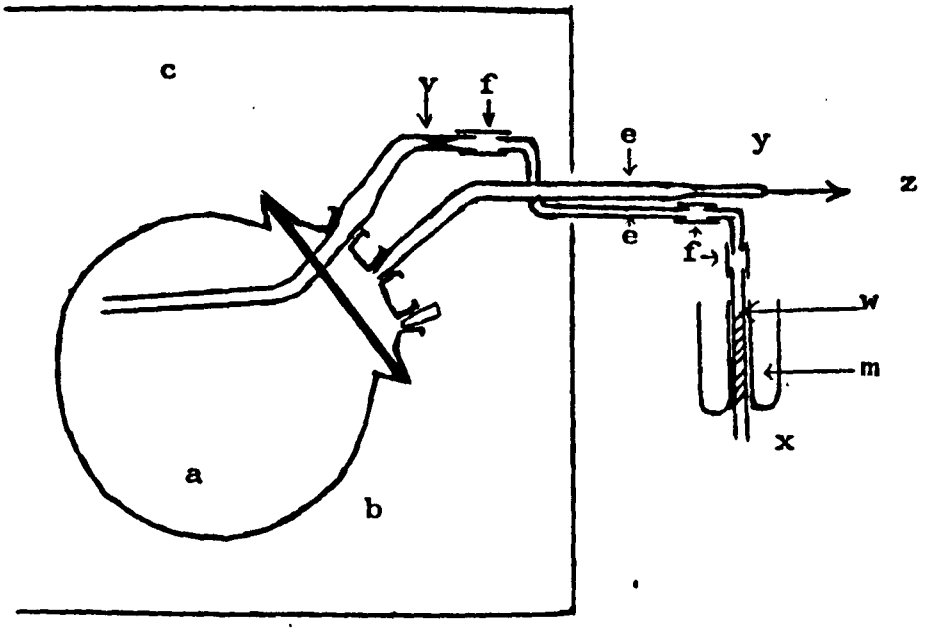
Fig.4: "Four X Sampling" (4 x S) System - Expt.2g.



Key:

a. Beef
b. 21 flanged flask
c. Oven
d. 1.5cm o.d. vent line
e. Glass tube
f. Polythene tubing
g. Glass T-junction
h. 50cm³ flask
i. Spiral condenser(42cm)
j. Glass manifold
k. Swagelok union(¼"/¼")
l. 1st sub-ambient Tenax tube(Sba 1)
m. Coolant trap
n. 2nd sub-ambient Tenax tube(Sba 2)
o. Rubber tubing
p. Drying tube
q. Flowmeter
r. Needle valve
s. 3/way tap
t. to atmosphere
u. to vacuum pump
v. Restricted vent line (capillary bore)
w. Sub-ambient external vent tube of Tenax
z. to condenser and remainder of sampling apparatus

Fig. 5: Diagram of Restricted Vent Line and Sba External Vent Tube - Expt.3d.



conditions were as in Expt. 2c.

3c: Experiments to Evaluate Vacuum Sampling.

Evidence from Expt. 3a had shown that contamination was not caused by adsorption of volatiles onto the oven walls. Similarities between chromatograms of blank oven and beef experiments pointed to two problems - excess water and the possibility of preferential sampling of oven headspace rather than cooked beef headspace. It was attempted to overcome the latter by sampling under vacuum.

(i) An experiment was performed as in Expt. 3a (i) with the following modifications. The flask contained 1kg. beef, and the flask vent line 'd' in Fig. 4 (p.33) (for pressure equalisation during cooking) was reduced to capillary dimensions. This was positioned in the flask during the cooking period and then replaced by a stopper just prior to sampling. A 5 min. period was allowed for the headspace gases to reform before sampling commenced at 500ml min^{-1}. Condenser water temperature and flow were controlled at 14°C and 1.2ℓ. min^{-1} respectively. All other experimental conditions were as for Expt. 2c.

(ii) The above experiment was repeated twice, initially with a sampling flowrate of 100ml min^{-1} and then at 300ml min^{-1}, instead of 500ml min^{-1}. In both cases, the period allowed for build-up of headspace gases was increased to 15 min.

3d: Assessment of Laboratory Atmosphere/Oven Headspace Contamination.

An experiment was performed as in Expt. 3a (i) except that (a) a Tenax tube containing 0.09g Tenax and acting as a scrubber was attached, by means of polythene tubing, to the restricted vent line and positioned outside the oven. This external vent tube was cooled in solid CO_2 (see Fig. 5, p.33). Its temperature was equilibrated as in Expt. 2c. (b) the vent tube and restricted line were in position during both cooking and sampling which took place at 500ml min^{-1} for 5 min. Condenser water and flow were controlled at 14°C and 1.2ℓ.min^{-1} respectively. All other experimental conditions were as for Expt. 2c.

3e: Systematic Elimination of Possible Sources of Contamination.

The results of Expt. 3d indicated that there was more than one possible source of contamination. A series of experiments was designed to locate these sources by successively eliminating the possible contribution made by individual components of the sampling system.

(i) Solid CO_2 contamination was tested for by capping one end of a Tenax tube (0.09g Tenax) and attaching the other end to 10ft. polythene tubing. The tube was equilibrated to -76.5°C for 30 min. in a solid CO_2-filled trap and then removed for GC analysis using the conditions described in Expt. 1a. and Expt. 2c.

(ii) The above procedure was repeated but using a 100ml min^{-1} dry N_2 flow via 50g silica gel, passing through the uncapped Tenax tube in order to test for solid CO_2 plus N_2 contamination.

(iii) Expt. 3e (ii) was repeated but using 50g molecular sieve 13X to replace the silica gel, to test once more for solid CO_2 plus N_2 contamination.

(iv) To test for solid CO_2 plus N_2 plus"clean" air contamination, a 5 min. sample of scrubbed high purity (HP) air from a cylinder (BOC Special Gases) was passed at 500ml min^{-1} through a Tenax tube, which had been equilibrated for 30 min. as described in (iii) above. The HP air supply was scrubbed by passing it through 50g molecular sieve 13X followed by a Sba Tenax trap (to simulate a Sba external vent tube). This had been equilibrated in a flow of N_2 purified by molecular sieve 13X.

(v) As in Expt. 3e (iv) above, but the 5 min. sample of scrubbed HP air was taken via the cooking flask in the oven at 205°C and the rest of the sampling apparatus in order to test for apparatus contamination in addition to the other possible sources described above. Condenser water temperature and flow were controlled at 14°C and 1.2L min^{-1} respectively.

3f: Experiments to Introduce a Scrubbed Air Supply for Heating and Sampling.

Evidence had shown that one source of contamination was the oven/laboratory atmosphere heated in the flask during cooking and drawn through the Tenax tubes, together with the cooked headspace, during sampling. It was therefore replaced by scrubbed HP air. Suitable changes in the sampling procedure were necessary.

(i) A blank oven experiment was performed as in Expt. 3e (v) with the following modifications. Immediately the flask was placed in the oven, scrubbed HP air was passed through it, via the external vent tube - in at position 'x' and out at 'y' in Fig. 5, (p.33) - at a rate of 500ml min^{-1} for 10 min. During this time the external vent tube coolant trap was filled with solid CO_2. After 10 min., the HP air cylinder was turned

off, the glass tube (e) capped at 'y' in Fig. 5, and the flask vented to atmosphere via the Sba external vent tube for the remainder of the heating period (total 1h.). After 30 min. the four Tenax sampling tubes were equilibrated to -76.5°C as described in Expt. 3e(iii). At the end of the heating period, the Tenax tubes were connected to the drying tubes and flow-meters, the flask connected to the sampling apparatus at 'y' in Fig. 5 (p.33) and a 50ℓ. Teflon bag (Chrompak Ltd.), previously filled with scrubbed HP air, connected with opened valve to the external vent tube at 'x' in Fig. 5 (p.33). The vacuum pump was used to draw over the flask headspace plus scrubbed air from the Teflon bag at 500ml min^{-1} for 5 min. through each sampling tube.

(ii) The above experiment was repeated but using an increased amount of molecular sieve (150g) on the HP air supply line.

3g: Experiments to Eliminate Glassware Contamination.

Eradication of any contamination due to the glassware within the oven was initially attempted by soaking and washing the glassware in various cleaning agents.

(i) Three blank oven experiments were performed as in Expt. 3f (ii) having soaked the oven glassware overnight in (a) RBS25 (Chemical Concentrates Ltd.), a bio-degradable chemically active surface agent, (b) concentrated HCℓ and (c) chromic acid. In each case, the glassware was rinsed in distilled water, then acetone followed by drying with scrubbed HP air.

(ii) A blank oven experiment was performed as in Expt.3f (ii) but using a new unused glass 2ℓ. flanged flask, to differentiate between the glass itself or the possibility of an accumulation of adsorbed volatiles as the cause of the glassware contamination.

(iii) A blank oven experiment was performed having first silylated the oven glassware by the addition of 1cm^3 Silyl-8 (Pierce Chemical Company) in 3cm^3 acetone (dried over molecular sieve 5A) to the flask, then heating it, connected to its various adaptors in the oven, for 2h. at 250°C in a 100ml min^{-1} stream of N_2 (dried and purified by 50g molecular sieve 13X). All other experimental conditions were as in Expt. 3f (ii).

3h: Experiments to Optimise the Flask 'Flushing' Procedure.

Evidence obtained pointed to the possibility that the rate of flow of the scrubbed HP air used for 'flushing' the laboratory atmosphere from the flask at the commencement of heating in Expts. 3f,g was too low. A blank oven experiment

was performed as in Expt. 3g (iii) except that scrubbed HP air was flushed through the flask at 2l. min^{-1} for 10 min. Additional purifying traps were used to ensure adequate scrubbing. These comprised a vastly increased amount of molecular sieve 13X (300g), which was regenerated prior to each experimental use, 200g silica gel (in a 70cm x 8cm o.d. glass column with the sieve) and a trap containing phosphorus pentoxide impregnated on glass wool to rigorously dry the large volume of air. All other experimental conditions were as for Expt. 3f (ii).

3i: Comparison of Venting Methods.

(i) A blank oven experiment was performed as in Expt. 3h using the external Sba vent tube for the heating period only. During sampling therefore, scrubbed HP air came directly from the Teflon bag. In this way, any volatiles which had formed during heating or which had originated from the HP air supply and collected on the external vent tube, could not be swept off during sampling and then adsorbed on the sample Tenax tubes.

(ii) The above experiment was repeated, replacing the external vent tube by a clean cooled Tenax tube for the sampling period.

(iii) During a blank oven experiment performed as in Expt. 3h (ii), the flask was connected to the sampling apparatus at position 'y' (Fig. 5, p. 33) immediately after flushing the flask with scrubbed HP air for 10 min. The flask was thereby vented during heating via the condenser and sampling Tenax tubes. The external vent tube was also replaced by a clean cooled Tenax tube for the sampling period as described in (ii).

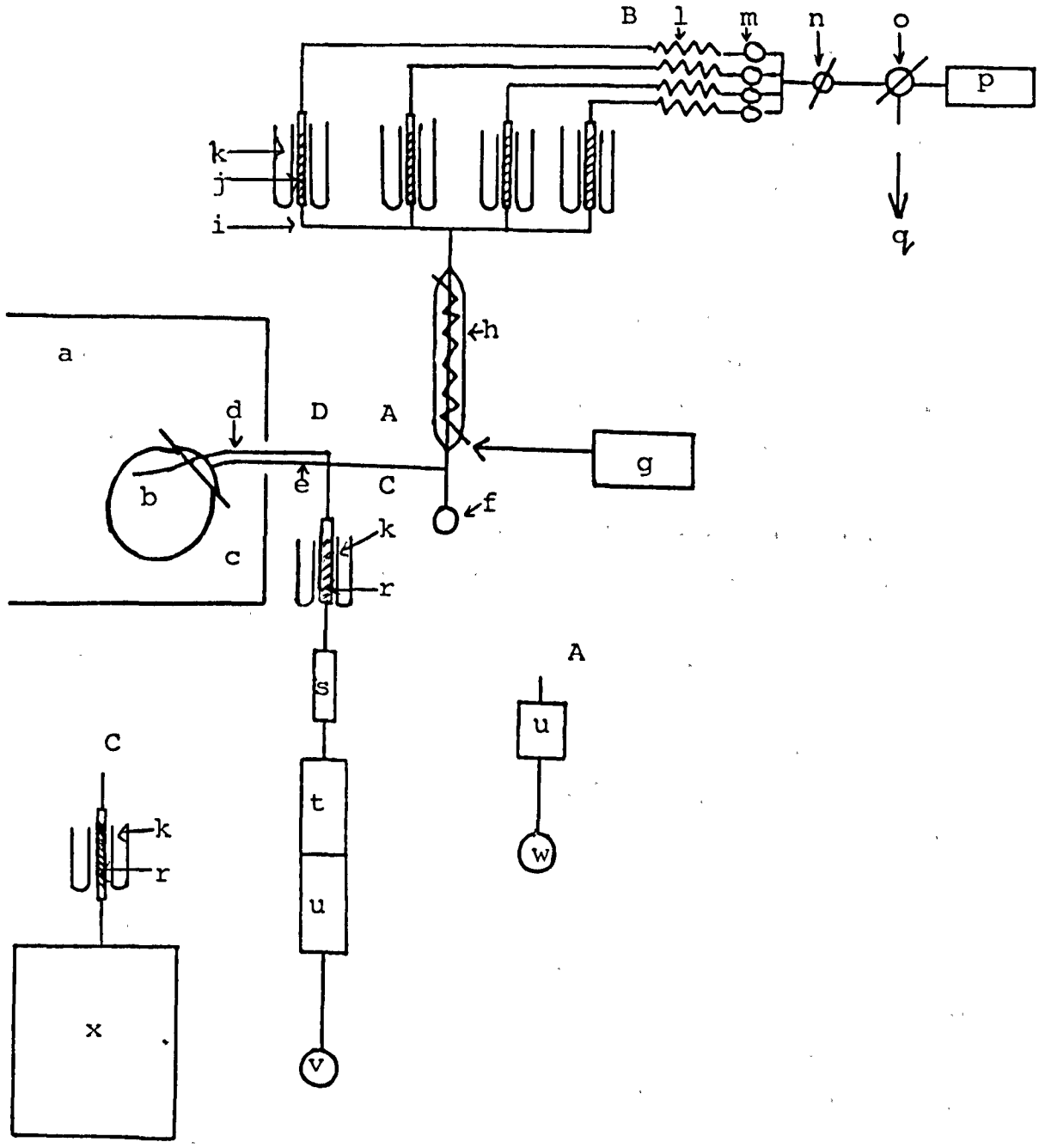
EXPERIMENT 4: Utilisation of the Optimised Sampling Method.

4a: Beef Experiments to Compare Venting Methods.

Two methods of venting the flask during heating gave satisfactory blank oven chromatograms. Ascertaining the better of the two was achieved by performing the appropriate beef experiments.

(i) 1kg topside, trimmed of all extramuscular fat, was cooked for 1h. at 205°C in the flask (c) of Fig. 6, (p.38). All other experimental conditions were as for Expt. 3i (ii) in which two Sba external vent tubes were used; one during cooking and the other during sampling.

Fig. 6: Diagram of Optimised Sampling Apparatus - Expt. 4b.



Key:

a. Oven
b. Beef
c. 21 glass flask
d. Restricted vent line
e. Glass tube
f. 50cm^3 flask
g. Water bath and circulating pump
h. 42cm spiral condenser
i. Glass manifold
j. Sub-ambient Tenax tube
k. Solid CO_2 coolant trap
l. Silica gel drying tube
m. Flowmeters
n. Needle valve
o. 3/way valve
p. Vacuum pump
q. to atmosphere
r. External vent Tenax tube
s. P_2O_5 drying trap
t. 200g silica gel
u. 300g molecular sieve 13X
v. HP air cylinder
w. N_2 cylinder
x. 50l. Teflon bag

(ii) Expt. 3i(iii) was repeated but with 1kg topside, trimmed of all extramuscular fat, cooked as above in the oven flask (c), (Fig. 6, p.38).

4b: The Optimised Sampling Method.

Using the apparatus depicted in Fig. 6, p.38, at time t=o disconnections were made at positions A and B. A 10ft. length of polythene was attached at B to each of the four Tenax (0.09g) tubes. The sampling apparatus was connected at A to the N_2 cylinder (w) attached to the molecular sieve 13X holder. A stream of dry, purified N_2 was passed through the sampling apparatus from A to B where the flow was measured to be 100ml min^{-1} through each of the four limbs. The water pump was turned on to circulate water at 14°C through the condenser at 1.2ℓ min^{-1} and the coolant traps filled with solid CO_2, then slotted over the 10ft. lengths of polythene tubing. This N_2 flow was continued for 30 min. (see below).

At t=20 min. the HP air cylinder (v), (connected to the column containing molecular sieve 13X and silica gel, the P_2O_5 drying trap and the external vent tube, all acting as scrubbers) was turned on. The flask, containing 1kg topside trimmed of all extramuscular fat, was placed in the oven at 205°C and connected to the external vent tube at position (C). The HP air flow was adjusted to 2ℓ min^{-1} (5 psi) and solid CO_2 was added to the coolant trap of the external vent tube. The scrubbed HP air flow from (v) to A was maintained for 10 min. (see below).

At t=30 min., the N_2 flow was disconnected at A and the glass tube (e) connected to the sampling apparatus. Immediately the air cylinder was turned off, disconnected at C and the polythene tubing connection at D closed with a Hoffman clip.

At t=80 min. (representing total beef cooking time of 1h), the sampling Tenax tubes were connected to the drying tubes and flowmeter at B. The 10ft. lengths of polythene tubing were removed. An unsampled Tenax tube was connected to the open-valved 50ℓ Teflon bag which had previously been filled with scrubbed HP air. The vacuum pump was switched into the sampling system at (o) and the needle valve (n) slowly opened whilst simultaneously releasing the Hoffman clip at D. The coolant trap of the clean replacement external vent tube was filled with solid CO_2 after adjusting all the flowrates to 500ml min^{-1}.

Sampling continued for 5 min. after which time the needle valve was closed and the 3/way valve (o) switched to atmosphere. The sampled Tenax tubes were then removed and capped ready for GC analysis, using the conditions described in Expt. 1a,(p.25) with the modifications introduced in Expt. 2c,(p.31). Solid CO_2 in the coolant traps was constantly replenished.

4c: Beef Experiments with Increased Cooking Periods.

A beef experiment was performed as in Expt. 4b using a cooking period of 4h. This was to obtain an "over-cooked" beef aroma sample. A cooking period of 2h was insufficient to achieve this.

EXPERIMENT 5: Sensory Validation of the Sampling Method.

5a: Sensory Validation Experiment of 1 x S System.

This experiment was performed at the stage of Expt. 2d (Amb, Sba 1, Sba 2 Tenax tubes) and Expt. 2f (Sba 1, Sba 2 Tenax tubes) so that any consideration of omitting the Amb tube in future experiments was justified (or otherwise) sensorially as well as on the basis of the gas chromatograms obtained (see p. 48, 52 + 53).

The gas chromatograph was fitted with a 30cm x 6mm o.d. empty glass column attached to an outlet splitter (Pye Unicam) of split ratio 10:1, - unity to the FID. A small glass funnel attached to the splitter outside the oven formed the odour port, and a small heater maintained at 225°C was located in the oven wall to prevent condensation of aroma components. Silylation of the column and splitter was achieved by the injection of 1µl Silyl-8(Pierce Chemical Company) onto the column (disconnected from the FID) maintained at 250°C with a 30ml min^{-1} flow of dry N_2 for 2h.

Two sets of Tenax tubes, sampled from beef experiments performed as in Expt. 2d (Amb, Sba 1, Sba 2 Tenax tubes, p.31) and as in Expt. 2f (Sba 1, Sba 2 Tenax tubes, p.31) were desorbed onto the GC column under the conditions described in Expt. 1a and modified in Expt. 2c. Four untrained but experienced assessors assessed the aroma eluting at the odour port and briefly described the perceived aroma for each of the 5 samples.

5b: Sensory Validation Experiment of 4 x S System.

The above experiment was repeated but desorbing a sample tube from the optimised 4 x S system experiment performed as

in Expt. 4b.

B. SEPARATION OF AROMA VOLATILES.

It was decided that the GC conditions described on p 25 and modified as on p. 31 could probably not be improved upon significantly. However, the GC heat desorption conditions were evaluated.

EXPERIMENT 6: Experiments to Optimise Heat Desorption Conditions.

The original heat desorption conditions had been chosen to achieve rapid transfer of volatiles from the Tenax tube to the GC column at a temperature in keeping with the cooking temperature used and the maximum operating temperature of Tenax. Several time/temperature combinations were assessed using samples obtained as in Expt. 2f. These were:-

(i) 250°C for 1 min.
(ii) 250°C for 30 min.
(iii) 300°C for 1 min.
(iv) 300°C for 30 min.

In place of the Energy Regulator a Variac controller was connected to the heater. This had been pre-calibrated with a thermocouple and pen-recorder to give the required temperature. When the selected temperature was reached, the sampled Tenax tube, connected to the GC column, was enclosed by the heater. One minute after reaching the required temperature inside the Tenax tube, (previously determined by thermocouple and pen recorder), the N_2 carrier gas was rediverted through the tube and the volatiles flushed onto the GC column. The Variac remained 'on' for the appropriate period in each case.

EXPERIMENT 7: Sensory Validation of Selected GC Analysis Technique.

It was aimed to evaluate whether the FID was analysing compounds which formed an aroma characteristic of cooked beef by assessing the aroma of a chromatographed sample trapped at the GC column exit.

A sampled Tenax tube obtained under the optimised experimental conditions of Expt. 4b was heat desorbed as described in Expt. 1a and as modified in Expt. 2c, onto a 5.5m x 6mm o.d. PEG 20M packed column. The column was attached to an outlet

splitter (ratio 100:1 to the detector) which had been silylated as described in Expt. 5a. The temperature of the GC wall heater was 225°C. The GC temperature program on p.25 commenced after heat desorption, and the eluant was trapped on a clean Tenax tube pre-cooled in solid CO_2. The collected total sample was then heat desorbed onto a 30cm x 6mm o.d. empty silylated glass column attached to a 100:1 splitter. Four untrained but experienced assessors described the perceived aroma as in Expt. 5.

EXPERIMENT 8: Quantification of Aroma Components.

Chromatograms obtained from the experiments in which the beef was cooked for 1h. (see Expt. 4b, p.39) and 4h. (see Expt. 4c, p.40) were used for the calculation of peak areas.

C. IDENTIFICATION OF AROMA VOLATILES.

EXPERIMENT 9: GC/MS Analysis of Aroma Volatiles.

9a: Development and Optimisation of a Pooling Technique.

In order to obtain samples in sufficient concentration for MS, sampled Tenax tubes were combined. This involved heat desorbing, under the conditions outlined and modified in Expts. 1b and 2c respectively, two Tenax tubes onto either a clean unsampled tube or onto each other. Using Tenax tubes sampled under the conditions of Expt. 2g, the procedures evaluated were:-

(i) heat desorbing two sampled Tenax tubes onto the same end of a clean unsampled tube,
(ii) heat desorbing two sampled Tenax tubes onto opposite ends of a clean unsampled tube,
(iii) heat desorbing one sampled tube onto the sampled end of another sampled Tenax tube.

All the sampled Tenax tubes were reversed for heat desorption onto other Tenax tubes or onto the GC column (excepting (ii)). Preliminary trials dictated a lowering of the N_2 pre-flush time of heat desorption during pooling to 5s in conjunction with the use of a solid CO_2-cooled Sba trap to cool the receiver Tenax tube to -76.5°C in a 30ml min^{-1} flow of dry N_2 for 10 min prior to pooling. This served to minimise the loss of low boiling components. Especially large amounts

of Tenax (0.114g) were used in the receiver tubes. This enabled pooling up to 16 tubes if necessary without exceeding the sampling capacity of the 'pooled' tube.

9b: GC/MS Analysis of Blank Experiment Sample.

GC/MS analysis was achieved by linking a GC (Perkin Elmer Sigma 3) via a heated all-glass jet separator interface to a mass spectrometer, Kratos MS25. The GC/MS conditions are outlined in Table 5 (other GC conditions as in Table 4, page 21).

Table 5: GC/MS Conditions of Analysis

GC	gas chromatograph	- Perkin Elmer Sigma 3 (modified by Kratos to link with MS)
	carrier gas	- Helium
	carrier flow rate	- 40ml min^{-1}
Interface	single stage all glass jet separator at 250°C	
MS	mass spectrometer	- Kratos, MS25
	source temperature	- 200°C
	trap current (EI)	- 100μA
	emission current (CI)	- 5mA
	ionisation potential	- 70eV (EI), 125eV (CI)
	mass range	- 15-800
	scan speed	- 1 - 3s/decade
	resolution	- nominal 600
Data System	Data General Nova 2 computer	
Software	Kratos DS50-S	

GC/MS analyses were performed on a Tenax tube from a 4 x S system blank experiment sampled under conditions of Expt. 3i(iii) by electron ionisation (EI) and chemical ionisation (CI, using methane as the reagent gas). This enabled identification to be as positive as possible when combined with information obtained from additional facilities such as scale expansion, retrospective background subtraction, manual deconvolution of unresolved GC peaks and mass fragmentograms. Verification and certainty of identification was greatly enhanced by the evidence of molecular weight obtained from CI.

9c: GC/MS Analysis ofAroma Isolate from Beef Cooked for One Hour.

GC/MS analyses, both EI and CI, were carried out as described in Expt. 9b on pooled Tenax tubes each resulting from pooling 8 sampled Tenax tubes from two 4 x S system experiments

performed as described in Expt. 4b. Pooling was as described in Expt. 9a(iii).

9d: GC/MS Analysis of Aroma Isolate from Beef Cooked for Four Hours.

GC/MS analyses, both EI and CI, were carried out as described in Expt. 9b on pooled Tenax tubes each resulting from pooling 4 sampled Tenax tubes from one 4 x S system experiment performed as described in Expt. 4c. Pooling was as described in Expt. 9a(iii).

RESULTS AND DISCUSSION

A. AROMA ISOLATION AND CONCENTRATION

EXPERIMENT 1: Development and Preliminary Trials of the Sampling Method

1a: Preparation of Tenax tubes and Blank Tenax Chromatograms

Blank Tenax chromatograms were virtually identical to the baseline obtained on temperature programming the GC column in the absence of a Tenax tube, under similar GC conditions. A typical blank Tenax chromatogram is presented in Fig.7.page45.

1b: The Initial Sampling System

The chromatograms obtained from Tenax tubes sampled at a flowrate of 150ml min^{-1} for 5,15,30 and 60 min respectively were promising. As sampling time increased, the number and intensity of peaks detected and also their resolution increased. Reproducibility on repeating the experiment was good. The best chromatogram (150ml min^{-1}/60 min is shown in Fig. 8 (page45).

1c: Blank Oven Experiment

Chromatograms obtained by repeating the above experiment in the absence of meat, showed numerous peaks. This suggested possible adsorption of volatiles onto the oven walls, followed by desorption during subsequent sampling, or possible contamination of the Tenax tubes by the vacuum pump exhaust. The latter was shown to be the prime cause and acceptable oven blank chromatograms were obtained when the experiment was repeated leading the pump exhaust vapours outside the laboratory. A typical oven blank chromatogram is shown in Fig. 9 (page45).

1d: Optimisation of Sampling Flowrate and Sampling Time

i) Chromatograms obtained from Tenax tubes sampled for 5, 15,30 and 60 min respectively for each of the flowrates 100, 125,175 and 250ml min^{-1} showed that for all the flows tested, increased sampling time caused increased GC background drift and consequent poor resolution.

probably due to increasing concentrations of water in the samples. This agrees with an effect previously reported, the drift being caused by water hydrogen bonding with polar volatiles in the sample and also with the PEG 20M GC column stationary phase[164]. The extent of drift was unacceptable for sampling times >15 min. The chromatograms shown in Fig.10 (page45) demonstrate no optimum flowrate in the range analysed, their overall strength increasing as flowrate increased. Thus, flows >250ml min^{-1} appeared desirable in association with a sampling time not >15 min.

ii) When sampling at 500ml min^{-1} for 15 min onto two Tenax tubes in series, the resulting chromatograms showed breakthrough from the first tube. This could be due to either an excessive flowrate or saturation of the Tenax in the first tube within the 15 min sampling period. Thus the sampling time was reduced to 5 min. Also a low sampling time was considered desirable, to obviate artefact formation during sampling. A flowrate of 500ml min^{-1} for 5 min, gives a total sample volume of 2.5ℓ from a 25ℓ capacity oven. Hence, a smaller container e.g. a 2ℓ flanged flask, within the oven would confine the volatiles before sampling. In addition, in view of the breakthrough problem, the second "in-series" ambient (Amb) tube was replaced by a sub-ambient (Sba) Tenax trap at -78°C (solid CO_2/acetone) thereby minimising breakthrough.

EXPERIMENT 2: Optimisation of the Sampling Method

2a: Attempts to obtain stronger chromatograms

i) Chromatograms obtained from Tenax tubes sampled at 500ml min^{-1} for 5 min with the beef cooked in a 2ℓ flanged flask were difficult to assess because the Amb tube chromatogram showed considerable background drift due to excessive water in the sample, whilst the Sba tube chromatogram was either an extremely strong one or else was significantly contaminated by acetone used in the coolant. It was also observed during sampling that water had collected in the right-angled bend (g) between the two Tenax tubes (see Fig. 3A, page30). When 5µl of this water were injected onto the GC column (see Table 4, p.27) only two peaks were detected - a water peak and one other. It was important that this water should not reach the Sba trap since, apart from causing chromatographic drift it would solidify and cause blockage.

ii) When the experiment was repeated, having modified the apparatus, as shown in Fig. 3B (page 30) and using a dessicant before the Sba tube, blockage of the Sba trap occurred, either during sampling or even before i.e. during the 30min sub-ambient temperature equilibration time. This indicated that even when water from the cooked beef headspace was not reaching the Sba trap, there was sufficient atmospheric water vapour in the Sba tube itself to block it during cooling and thereby prevent sampling. This was prevented in future experiments by equilibrating the Sba tube for 30 min in a dry N_2 flow.

During this experiment difficulties arose when the vacuum was switched into the sampling sysytem via the 3/way valve (k). The Tenax was sucked out of the tubes and sampling could not be continued. A needle-valve in front of the 3/way valve was therefore introduced to enable the vacuum to be switched in more gradually.

The dessicant K_2CO_3 was selected since Farrington et al. had estimated the recoveries of 5 different drying agents and found that K_2CO_3 gave 100% recovery for all compounds tested except isopropyl alcohol[165]. Kato recommended the use of finely granulated K_2CO_3 rather than the powder form for removing water[166].

2b. Experiments to Limit the Amount of Water Being Sampled.

Chromatograms obtained from Tenax tubes sampled when a vertical water condenser was inserted before the Tenax traps gave very promising results. These are shown in Figs. 11A,B (page 48). Both the Amb and Sba tube chromatograms show good resolution of peaks, although there is some baseline drift; however this is not the "splurge" effect associated with excess water in the samples. The main difference between the two chromatograms is that the Amb tube has adsorbed more of the water and higher boiling components whereas the Sba tube has retained the lower boiling components which have "broken through" the Amb tube.

Approximately $30cm^3$ of water collected at the base of the condenser and when 5µl of this were injected onto the GC column (see Table 4.p.11) no detectable peaks other than the water peak and slight drift were observed.

The needle valve, inserted beyond the Sba trap (i) (page 30) to allow a gradual increase in vacuum during sampling, functioned successfuly as hoped. Tenax was no longer sucked

out of the tubes on commencement of sampling. Equilibrating the Sba tube in dry N_2 also proved successful, as blockage of the Sba trap by solidification of atmospheric water vapour did not occur.

Results showed that the amount of water which could be tolerated chromatographically was critical. Excessive amounts obscured organic volatiles on the resulting chromatograms. On the other hand complete removal of water brought about a concurrent excessive removal of organic volatiles. A suitable compromise was achieved in this experiment. Although the main restriction was to keep the dead-volume between the beef-containing flask and the Tenax tubes to a minimum, the introduction of the water condenser and small flask at its base was not detrimental. On the contrary, much improved samples were obtained. All subsequent experiments were therefore based on developing and standardising this modification.

2c. Experiment to Prevent Coolant Contamination

The chromatograms obtained are shown in Figs. 12A,B (page 48). The new coolant trap design in conjunction with the use of solid CO_2 alone as coolant and Swagelok unions for connection of the Tenax tubes provided a great improvement over Expt. 2b. These modifications were also more practicable and were therefore retained.

The GC modifications also proved successful and were retained in future experiments. Reducing the GC N_2 flush time to 3.5 min achieved most peaks appearing on the chromatogram after the temperature program started. The benefits of raising the final temperature to 175°C were not assessed in detail but the modification was retained in view of the logic behind its introduction.

2d: Experiment to Determine the Efficiency of the Adsorbent Traps.

Chromatograms obtained from the use of two sub-ambient traps (Sba 1 and Sba 2) in series with an Amb Tenax tube showed that some volatiles were passing through the first two tubes and being adsorbed by Sba 2. The Amb and Sba 1 chromatograms were very comparable to their counterparts obtained in Expt. 2c (Figs. 12A,B); the Sba 2 chromatogram is shown in Fig. 13 , page 48 . The extent of breakthrough from the Sba 1 trap was sufficiently great to decide that the

Sba 2 trap be used for sampling in future experiments.

2e: Experiment to Determine Optimum Sampling Flowrate and Sampling Time.

The three sets of chromatograms obtained from sampling at different flowrate/time combinations onto Amb, Sba 1 and Sba 2 Tenax tubes in series were compared with those described in Expt. 2d above (500ml min^{-1} for 5 min) i.e. Figs.12A,B, page 48 (Amb and Sba1) and Fig. 13 , page 48 (Sba2). The aim was to obtain strong chromatograms for Amb and Sba 1 and to minimise any breakthrough onto Sba 2.

(i) The Amb and Sba 1 chromatograms resulting from tubes sampled at 500ml^{-1} for 10 min and shown in Figs.14A ,B , page 51 showed that some slight improvement was achieved with respect to peak intensity. However undesirable breakthrough onto Sba 2 (Fig.14C), was also increased. Thus, under present conditions, drawing through twice the volume of cooked beef headspace achieved little, and so the previous 5 min sampling time was not too short.

ii) Amb and Sba 1 Tenax tubes sampled at 250ml min^{-1} for 5 min gave a set of chromatograms of slightly lower peak intensities (Figs.15A,B). Chromatographic resolution also suffered. However there was very little real difference between the corresponding Sba 2 chromatograms (see Fig.13 and Fig.15C); thus breakthrough of volatiles from the Sba 1 trap was not decreased by sampling at half the flowrate. Achieving strong Amb and Sba 1 chromatograms was a priority and therefore the conditions of Expt. 2d were preferable.

iii) Chromatograms obtained from tubes sampled at 250ml min^{-1} for 10 min (Figs.16A,B,C)showed that the Amb chromatogram was very comparable with that obtained from the same volume of cooked headspace sampled more quickly in Expt. 2d. However the Sba chromatograms showed slightly more intense peaks than the corresponding chromatograms of Expt. 2d.

Bearing in mind that the aim was to capture the aroma quickly, a short sampling time was desirable in practice. In view of this it was decided to retain the conditions of sampling at 500ml min^{-1} for 5min. in future experiments.

2f: Experiment to Determine the Minimum Number of "in series" Adsorbent Traps for sampling.

Chromatograms resulting from the use of two Sba traps only, and shown in Figs. 17A,B(p.52) were carefully compared

with those described in Expt. 2d in order to assess the possibility in future of omitting the Amb trap. The high boiling components (preferentially trapped on Amb in Expt. 2c) and also the low boiling components (preferentially trapped on Sba 1 of Expt. 2c) were represented in the Sba 1 chromatogram of this experiment. This showed that many of the peaks previously adsorbed by the Amb trap were collected by the Sba 1 trap when used in the absence of an Amb tube.

However the decision to retain two Sba traps only for sampling in future depended on whether the aroma collected on said tubes was truly representative of roast beef aroma. This was the case (see Expt. 5a for the sensory validation experiment). Thus the omission of Amb was justified. The Sba 2 chromatogram of this experiment (see Fig.17B) was slightly stronger than that of Expt. 2d (see Fig.13 , p.48), but this effect, probably caused by saturation of the Sba 1 Tenax trap, was not very significant. The combination of two Sba traps for sampling was thus standardised on in future experiments.

2g: Experiment to Increase the Number of Samples from Each Beef Experiment.

Typical Sba 1 and Sba 2 chromatograms obtained from the 4 x S system of Fig. 4 (p.33) showed high intensity peaks and little drift. These are shown in Figs. 18A,B, p.52. The large 42cm spiral condenser was adequate to prevent excess water reaching the Sba tubes and therefore coped with the increased flow at this point in the 4 x S system.

Comparing the chromatograms obtained from the 1 x S system in Expt. 2f (see Figs.17A,B p.52) with those obtained from the 4 x S system in this experiment (see Figs.18A,B), the 4 x S chromatograms were slightly weaker. It was checked that a sample from a 4 x S system was sufficiently concentrated for sensory analysis by the method described in Expt. 5a. Reproducibility within each set of chromatograms from four equivalent Tenax tubes was good. The main asset of the 4 x S system was to obtain four identical samples from each 1kg. beef, thereby reducing costs and increasing the number of replicate samples for future chemical and sensory analyses.

2h: Evaluation of Tenax Capacity.

The Sba 1 chromatograms resulting from the 4 x S system

experiment in which the sample tubes contained twice the amount of Tenax showed high intensity peaks which were also well-resolved (Fig.19A, p.52). The chromatograms of the corresponding Sba 2 'double Tenax' tubes (Fig.19B) showed no peaks whatsoever. Hence breakthrough of volatiles from the Sba 1 trap did not occur. This showed, that under these conditions, the flowrate was not excessively high and that the Tenax in Sba 1 had not been saturated. Hence it was decided to omit the Sba 2 trap whilst retaining doubled amounts of Tenax in the one used.

EXPERIMENT 3: Blank Experiment.

3a: Initial Blank Oven Experiments.

(i) A typical chromatogram resulting from a Sba Tenax tube sampled in the absence of beef, and shown in Fig.20A (p.55) suspiciously resembled that described in Expt. 2h (see Fig.19A, p.52). The problem was two-fold; namely excess background drift caused by water, and the presence of contaminant peaks from some unknown source(s). The latter suggested, in view of the similarity between chromatograms described above, the possibility of beef volatiles being adsorbed on the oven walls, subsequently desorbed by oven heat during the next experiment, and then sampled on the Tenax tubes.

(ii) The chromatograms obtained after thoroughly scouring the oven walls to remove any accumulation of volatiles showed no reduction in either background drift or in the intensity of the peaks present. It was unlikely therefore that adsorption onto and desorption from the walls had any part in the unacceptable blank oven experiments. Another possible source of these contaminants was the oven headspace which was drawn through the Tenax tubes during sampling i.e. effectively acting as an entraining gas. It was possible that preferential sampling of oven headspace rather than beef headspace was occurring.

3b: Experiments to Determine Efficiency of the Water Condenser.

None of the chromatograms obtained from the experiments using constant condenser water temperatures of 14.5°, 12° and 8°C respectively showed a reduction in the level of background drift. Despite the evidence obtained, it seemed a wise precaution to incorporate the control of condenser water temperature and flowrate (1.2 $\ell.min^{-1}$) into the sampling procedure

in future. A temperature of 14°C was selected, as not differing greatly from previous experimental practice. It was next attempted to prevent the possibility of preferential sampling of oven headspace over cooked beef headspace.

3c: Experiments to Evaluate Vacuum Sampling.

(i) Practical difficulties arose during sampling when the vent line was restricted to capillary size for cooking and replaced by a stopper for sampling under vacuum at an initial flow of 500ml min^{-1}. Sampling had to be stopped after 2 min. due to violent refluxing of water in the condenser. The resulting chromatogram was swamped with water. The total sampled volume of 10ℓ. of cooked beef headspace was therefore impracticable under these conditions and it was decided to reduce the sampling flowrate to 100ml min^{-1} [2ℓ.(flask volume)÷ 4 (no. sample tubes in parallel) ÷ 5 (sampling time, min.) = 100ml min^{-1}]

(ii) The chromatograms obtained from Tenax tubes sampled at 100ml min^{-1} for 5 min. were very weak, probably due to insufficient vacuum being achieved within 5 min. An initial flow of 300ml min^{-1}, which provided adequate vacuum without causing violent refluxing, gave chromatograms showing high levels of background drift. Thus it was more practicable to use the restricted vent line for both cooking and sampling.

3d: Assessment of Laboratory Atmosphere/Oven Headspace Contamination.

The blank oven chromatogram, resulting from the experiment in which the restricted vent line and external vent tube had been used for cooking and sampling showed some reduction in the intensities of the contaminant peaks (see Fig.21A, p.55). The Sba external vent tube chromatogram (see Fig.21B) suggested that much of the chromatogram described in Expt. 2h (Fig.19A, p.52) could have indeed been due to laboratory atmosphere. At this point, it was concluded that the Sba external vent tube was adsorbing volatiles which would otherwise have been drawn into the flask and collected on the Tenax tubes during sampling. It was therefore retained in future experiments.

3e: Systematic Elimination of Possible Sources of Contamination.

(i) The chromatogram obtained from the Tenax tube which had equilibrated in solid CO_2 for 30 min. was identical to a blank Tenax chromatogram e.g. see Fig. 7 (p.45). This proved that

under these conditions no contamination arose from the CO_2 itself.

(ii) The chromatogram resulting from the tube equilibrated to -76.5°C in a flow of N_2 dried via silica gel showed considerable contamination. From Fig.22A (p.55) it can be seen that a major source of contamination had been located, namely the N_2 supply.

(iii) The chromatogram of the tube equilibrated to -76.5°C in a flow of N_2, which had passed through molecular sieve 13X instead of silica gel, is shown in Fig.22B. The relative absence of peaks demonstrates that a source of contamination had been eliminated. The molecular sieve was both efficient in use and sufficient in quantity. Therefore it was incorporated in future experiments.

(iv) The 5 min. scrubbed HP air sample gave the chromatogram shown in Fig.22C. Virtually no peaks were evident. Hence contamination did not arise from scrubbed HP air when sampled at ambient temperatures.

(v) The chromatogram resulting from the Tenax tube which had taken a 5 min. scrubbed HP air sample via the hot flask in the oven and the rest of the sampling apparatus beyond the oven showed gross contamination (Fig.22D). Thorough cleaning of the oven glassware followed by rinsing in acetone made just a slight reduction in the amount of contamination.

It therefore remained to conclude that contamination was due to oven/laboratory atmosphere heated in the flask during cooking and/or compounds adsorbed onto the glass inside the oven, which were then released by heat but not removed by acetone. Contamination from the N_2 supply used during temperature equilibration of the Sba Tenax tubes had been successfully eliminated, by passing it through 50g molecular sieve 13X.

3f: Experiments to Introduce a 'Scrubbed' Air Supply for Heating and Sampling.

(i) The blank oven chromatogram obtained from sampling with scrubbed HP air from a Teflon bag, (having first replaced the laboratory atmosphere in the oven flask by scrubbed HP air from a cylinder) is shown in Fig.23, p.58. Although no background drift was apparent, contamination remained. This may have been due to insufficient purification of the HP air.

(ii) Increasing the amount of molecular sieve on the HP air supply line to 150g resulted in chromatograms very comparable to that obtained above. Thus an adequate amount of molecular sieve

had probably been used in the above experiment. A distinct benefit of this procedure was the reduction of background drift to tolerable levels. Obviously water vapour in the atmosphere contained by the oven flask had been present in sufficient amounts at oven temperature to cause excessive background drift. Thus, the drift part of the blank oven problem had been overcome.

It was extremely difficult to assess the value of the scrubbed air used to flush the laboratory atmosphere from the flask and also for sampling because the different contributors to the total contamination problem could not be individually dealt with. However, having taken all precautions to minimise contamination arising from the effect of heat on laboratory atmosphere within the oven flask, and assuming them to be effective, it remained to eliminate the final contribution made by the oven glassware. The procedures of flushing the flask and later sampling with scrubbed HP air were used in future experiments.

3g: Experiments to Eliminate Glassware Contamination.

(i) The chromatograms obtained from Tenax tubes sampled in blank oven experiments in which the glassware had been soaked in (a) RBS25, (b) conc. HCl and (c) chromic acid respectively showed virtually no reduction in contamination. The cleaning operation had been designed to remove residues of accumulated adsorbed volatiles. It was now thought possible that the contamination might be caused by the glass itself. This was assessed by using a new, un-used flask.

(ii) The chromatograms resulting from the blank oven experiment employing a new glass flask showed some improvement. However, contamination was still significant and unacceptable. This evidence pointed conclusively to the glass itself, at oven temperatures, as the prime cause of the contamination. It was sought to overcome this problem by silylating the flask and adaptors before use. Silylation involves the introduction of a trimethylsilyl group - $Si(CH_3)_3$ into a molecule to substitute for an active hydrogen, thereby reducing the polarity of the reactant and lowering the possibilities of hydrogen-bonding. Thus, by decreasing the number of reactive sites with active hydrogen, stability of the silylated product is enhanced[167].

(iii) The blank oven chromatogram obtained from the tube sampled

using a silylated flask is shown in Fig.24A (p.58). The results were extremely good: the chromatogram showed no background drift and a marked reduction of contamination peaks. It was decided that silylation of the flask and adaptors provided a satisfactory working blank oven chromatogram.

Resilylation of the flask and adaptors between each beef experiment was necessary because cleaning removed/inactivated the silylating agent and silyl derivatives which are rapidly decomposed by water to form siloxanes[168].

A blank oven experiment was also necessary before each beef experiment in order to monitor the amount of background contamination present.

Unfortunately the blank oven chromatograms were not always reproducible nor satisfactory (e.g. see Fig.24B). As it had been shown previously that much of the contamination had been due to heating laboratory atmosphere within the flask, it was thought that this variability might have been due to inefficient replacement of laboratory atmosphere in the flask by scrubbed HP air. Flushing the flask at the commencement of the heating period was believed to be beneficial in reducing laboratory atmosphere contamination; it was thought possible that the rate of 'flushing' was critical.

3h: Experiments to Optimise the Flask 'Flushing' Procedure.

(i) Increasing the scrubbed HP air flushing rate to $2\ell.min^{-1}$ for 10 min. gave a blank oven chromatogram showing a slight improvement (Fig.25 p.58). This was deemed to be the genuine background occurring under the conditions of heating an empty 2ℓ. flanged flask for 1h at 205°C and incorporating all the precautions described in Expts. 3a to 3g(iii). Experience showed that this rate of flushing proved critical and the increased flowrate was retained for future experiments. However, it was equally critical to maintain the adequacy of purification of the air supply with confidence, using the rigourous procedures described on p.37. The molecular sieve had to be re-generated immediately prior to each experimental use. Fresh P_2O_5 traps were made when necessary.

3i: Comparison of Venting Methods.

(i) Removing the external vent tube before sampling, during a blank oven experiment, resulted in the vent tube chromatogram shown in Fig.26A,p .58. When compared with (a) the equivalent

blank oven chromatogram depicted in Fig. 26B and (b) Fig. 25 p.58, it is clear that compounds trapped on the external vent tube were being swept off during sampling. The peaks in Fig.26A represent the contamination formed during the heating period despite the precautions taken. It was hoped that, by using a clean, cooled external vent Tenax tube during the sampling period, the problem would be solved.

(ii) The blank oven chromatogram obtained when different external vent tubes were employed for heating and for sampling is shown in Fig.26C. A distinct improvement can be observed (see Fig.26B). Thus, contaminants trapped during heating were effectively removed and further purification of the Teflon bag air supply during sampling was also achieved. This modification was employed in all further experiments.

(iii) By venting via the sample Tenax tubes during heating, the resulting blank oven chromatogram (Fig.26D) showed slightly more contamination than that obtained above. This was to be expected since contaminants formed during heating were in this case adsorbed onto the sample Tenax tubes during the heating period.

Either method of venting - via the Sba vent tube or via the sampling Tenax tubes - gave satisfactory blank oven chromatograms. Although the latter showed rather more background, this system did have the advantage that in a beef experiment, any headspace volatiles formed during heating would collect on the sample Tenax tubes, rather than be collected on the subsequently removed external vent tube.

An acceptable level of background contamination had been achieved. Thus it remained to assess chromatograms resulting from beef experiments using the latter two different venting methods and other successful modifications achieved in this Blank Oven Experiment.

EXPERIMENT 4: Utilisation of the Optimised Sampling Method.

4a: Beef Experiments to Compare Venting Methods.

(i) The beef experiment in which the flask had been vented during heating via the Sba external vent tube gave a chromatogram showing strong peaks although the resolution was not particularly sharp.

(ii) The chromatogram obtained from a beef experiment in which venting during heating was achieved via the sample Tenax tubes

beyond the water condenser, showed little background drift. Many large peaks were evident in the higher boiling region and the resolution was good.

A comparison of the two chromatograms showed that venting via the sample tubes was preferable. The experimental conditions also had several advantages -

(a) Volatiles given off during heating were collected on the sample Tenax tubes.

(b) Practical difficulties associated with Expt. 4a (i) were avoided, e.g. in Expt. 4a (i) the drip exuded by the cooked beef tended to reach the Sba external vent tube and block it by solidifying. In turn this created a build-up of pressure in the flask. Thus future utilisation of the optimised and standardised sampling procedure involved venting during heating via the sample Tenax tubes.

4b: The Optimised Sampling Method

The chromatograms obtained from the optimised sampling procedure using the apparatus depicted in Fig. 6 , p. 38 are shown in Fig.27A,B,C,p. 63 . Gas chromatograms were obtained using different GC attenuation settings in order to achieve all peaks on-scale. Further analysis and discussion of these is presented in Expts. 8, 9 and 15.

4c: Beef Experiments with Increased Cooking Periods.

Cooking beef as described in Expt. 4b for 4h resulted in the chromatograms shown in Fig.28A,B,C,D,p.64.It can be seen that when compared with the chromatograms of Expt. 4b (1h cooking period), a very considerable increase in peak intensities and changes in peak pattern had been achieved. Further analysis and discussion of these results is presented in Expts. 8, 9 and 15.

EXPERIMENT 5: Sensory Validation of the Sampling Method.

5a: Sensory Validation Experiment of 1xS System

Odour descriptions of sampled Tenax tubes from Expts. 2d and f showed three distinct stages - see Table 6, p.65. These correspond to the 3.5 min N_2 pre-flush, heating (2 min) and the N_2 flush-on stages of heat desorption.

It can be seen that, sensorially, the aroma collected on the Amb, Sba 1 and Sba 2 Tenax tubes of Expt. 2d was also collected on the Sba 1 and Sba 2 Tenax tubes of Expt. 2f, thereby justifying the omission of the Amb trap from Expt. 2f

Table 6: Aroma Descriptions of Desorbed Tenax Tubes from the 1 x S System and Perceived at the GC Odour Port

Tenax tubes (0.045g)	Heat Desorption Stage	Samples from Expt.2d Aroma Description	Samples from Expt.2f Aroma Description
Amb	pre-flush	sickly, estery, slightly fatty	
	heating	dull, meaty, slightly musty	
	flush-on	meaty, roast beef, typical aroma of roast beef obtained on cooking	-
Sba 1	pre-flush	meaty, sweet, very pleasant	weakly meaty, fatty, broth-like, boiled beef aroma
	heating	-	nutty, musty
	flush-on	typical roast beef aroma of cooking, very slightly charred, burnt	typical roast beef aroma obtained on cooking
Sba 2	pre-flush	slightly solvent-like, estery	weakly meaty, brothy, slightly sulphurous
	heating	fatty, slightly rancid aroma	-
	flush-on	strong, meaty, Bovril, roasted, burnt, rubbery	slightly burnt, smoky, charcoal- steak aroma, roasted meat, Bovril, Marmite

onwards. In particular, the characteristic roast beef aroma was definitely present.

5b: Sensory Validation Experiment of 4xS System

Odour qualities of a desorbed Tenax tube sampled as in the optimised Expt. 4b are presented in Table 7.

Table 7: Aroma Descriptions of a Desorbed Tenax Tube from the 4xS System and Perceived at the GC Odour Port.

Sba Tenax (0.09g) tube	Heat Desorption Stage	Aroma Description
Expt. 4b	N_2 pre-flush	sweet, sickly, diacetyl-like, buttery, meaty, boiled beef aroma, oily/fatty, musty.
	Heating	-
	N_2 flush-on	earthy, charred, smoky, burnt, toasted, pungent and roasted cereal-like at first, culminating in the typical roast beef aroma obtained on cooking

This experiment therefore validates both the sampling and desorption procedures in that it shows that the Tenax is adsorbing and desorbing volatiles which give cooked beef its aroma (under the analytical conditions employed).

B. SEPARATION OF AROMA VOLATILES

EXPERIMENT 6: Experiment to Optimise Heat Desorption

The required temperature could not be maintained for long periods with any degree of accuracy using the energy regulator. The Variac-incorporated method was developed for this experiment. All combinations tested gave chromatograms which, when compared with their counterparts obtained from sample tubes heat desorbed at 250°C/1 min using the energy regulator, showed peaks of lower intensities particularly for the relatively high boiling components. This may have been due to the difference in heat desorption technique. For example, use of the Variac controller required 8 min for the Tenax inside the sample tube to reach 250°C. The impact of a rapid transfer of volatiles was thus considerably lessened.

It was concluded that the energy regulator, which permitted the interior of the Tenax tube to reach 250°C in one minute for heat desorption conditions of 250°C for 1 min, was vital for optimum heat desorption, which in turn gave strong and well-resolved chromatograms.

EXPERIMENT 7: Sensory Validation of the Selected GC Analysis Technique.

Table 8 describes the aroma perceived when a once-chromatographed and collected total sample was heat desorbed into a short empty GC column.

Table 8: Aroma Descriptions Perceived at GC Odour Port of a Total Sample Collected at the GC Column Exit.

	Heat Desorption Stage	Aroma Description
Sba Tenax (0.09g) tube collected chromatographed total sample - Expt. 7	N_2 Pre-flush	sweet, buttery, flat, dull, musty, meaty, boiled beef aroma
	Heating	-
	N_2 Flush-on	earthy, charred, smoky, burnt, toasted, pungent, roasted beef aroma, sweet.

This evidence shows that a good cooked beef aroma sample had been collected at the GC column exit after eluting through the 5.5m PEG 20M packed column. The odour descriptions are very similar to those presented in Table 7 for the original

total sample (uncollected). Thus, any possible changes occurring during chromatography were insignificant. The FID is therefore receiving genuine cooked beef aroma components. Furthermore, an effcient trapping procedure had been developed.

EXPERIMENT 8: Quantification of Aroma Components.

The peak areas (mm^2) have been normalised to an attenuation of 5×10^2 (5×10^{10} A FSD). These are tabulated for the 1h and 4h cooking experiments (Expts. 4b and 4c) in Tables 9 and 10 respectively. A relative percentage abundance (RPA) figure has been calculated for each peak as follows:-

$$RPA = \frac{\text{peak-area}}{\text{total peak area}} \times 100$$

C. IDENTIFICATION OF AROMA VOLATILES

EXPERIMENT 9: GC/MS Analysis of Aroma Volatiles.

9a: Development and Optimisation of a Pooling Technique.

Comparison of the results of (i) and (ii) in which 2 sampled Tenax tubes were heat desorbed onto the same end (i) or opposite ends (ii) of a clean unsampled Tenax tube, showed stronger chromatograms for (i). By pooling one Tenax tube onto another sampled Tenax tube (iii), the total number of heat desorptions was decreased and the resulting chromatograms showed overall increased peak intensities. Generally, most polymer traps have been successively heat desorbed into cryogenic traps prior to GC and/or GC/MS analysis[83,125]. The technique described above represents a novel approach which remains in keeping with the heat desorption method originally developed for rapidly transferring the adsorbed aroma volatiles to the GC column.

9b: GC/MS Analysis of Blank Experiment Sample.

The peaks identified from a typical blank experiment sample (Fig. 26D, p. 58) are listed with their mass spectra in Table 11.

Mass spectra were interpreted by comparison with a standard text of mass spectral data[168], and many other literature mass spectral data of food aroma components, compiled in these laboratories.

The sources of the compounds identified are straight-

Table 9 : Peak Areas for 1h Aroma Isolate

Peak No	Absolute area (mm^2)	Relative Percentage Area [a]	Peak No.	Absolute area (mm^2)	Relative Percentage Area [a]
1	12	0.02	47	2070	4.25
2	3	-	48	127	0.25
3	3	-	49	105	0.20
4	126	0.25	50	10	0.02
5	220	0.50	51	100	0.20
6	-	-	52	1158	2.50
7	12	0.02	53	585	1.25
8	5208	11.0	54	125	0.25
9	-	-	55	44	0.10
10	648	1.25	56	194	0.50
11	954	2.00	57	4672	10.00
12	666	1.50	58	39	0.10
13	-	-	59	6	0.01
14	396	0.75	60	150	0.25
15	12360	26.00	61	325	0.50
16	-	-	62	229	0.50
17	2640	5.50	63	536	1.25
18	56	0.10	64	20	0.05
19	128	0.25	65	195	0.50
20	3250	7.00	66	136	0.25
21	-	-	67	263	0.50
22	-	-	68	50	0.10
23	72	0.20	69	37	0.10
24	1712	3.50	70	217	0.50
25	16	0.02	71	9	0.02
26	840	1.75	72	317	0.75
27	1152	2.50	73	131	0.25
28	48	0.10	74	-	-
29	-	-	75	125	0.25
30	124	0.25	76	25	0.05
31	360	0.75			
32	6	0.01	Total Peak Area	47784	101.00
33	288	0.50			
34	144	0.25			
35	371	0.75			
36	460	1.00			
37	45	0.10			
38	19	0.05			
39	15	0.02			
40	787	1.25			
41	645	1.25			
42	20	0.05			
43	60	0.10			
44	4	0.01			
45	154	0.25			
46	1760	3.75			

Footnote: [a] RPA figures have been corrected as follows:-
> 5% quoted to nearest 0.5%
0.25 - 5% quoted to nearest 0.25%
< 0.25% quoted as 0.25, 0.20, 0.10, 0.05, 0.02, 0.01

"-" denotes no accurate peak area measurement possible although trace amount present.

Table 10: Peak Areas for 4h Aroma Isolate

Peak No.	Absolute area (mm^2)	Relative Percentage Area[a]	Peak No.	Absolute area (mm^2)	Relative Percentage Area[a]
1	93	0.10	42	140	0.10
2	1032	1.00	43	352	0.25
3	816	0.75	44	-	-
4	-	-	45	390	0.50
5	7950	7.50	46	-	-
6	990	1.00	47	888	0.75
7	-	-	48	-	-
8	-	-	49	576	0.50
9	3160	3.00	50	-	-
10	1064	1.00	51	72	0.05
11	-	-	52	288	0.25
12	1232	1.25	53	-	-
13	2376	2.25	54	88	0.10
14	2856	2.75	55	300	0.25
15	7630	7.00	56	80	0.10
16	-	-	57	10,400	10.00
17	816	0.75	58	609	0.50
18	72	0.05	59	68	0.75
19	5830	5.50	60	525	0.50
20	31,500	30.00	61	198	0.20
21	828	0.75	62	323	0.25
22	300	0.25	63	550	0.50
23	160	0.20	64	920	1.00
24	1995	2.00	65	189	0.20
25	1196	1.25	66	82	0.10
26	216	0.20	67	315	0.25
27	180	0.20	68	8	0.01
28	1278	1.25	69	90	0.10
29	1248	1.25	70	-	-
30	7280	7.00	71	247	0.25
31	208	0.20	72	70	0.05
32	80	0.10	73	270	0.25
33	-	-	74	-	-
34	1854	1.75	75	77	0.05
35	-	-	76	128	0.10
36	672	0.75	77	119	0.10
37	742	0.75	78	22	0.02
38	416	0.50	79	300	0.25
39	96	0.10	80	70	0.05
40	520	0.50	81	-	-
41	320	0.25			
			Total Peak Area.	105760	101.48

Footnote: [a] RPA figures have been corrected as follows:
> 5% quoted to neatest 0.5%
0.25 - 5% quoted to nearest 0.25%
< 0.25% quoted as - 0.25, 0.20, 0.10, 0.05, 0.02, 0.01

"-" denotes no accurate peak area measurement possible although trace amount present.

forward. Acetone arises from its use as a diluent for the silyating agent whereas trimethylsilanol forms as a product of hydrolysis of the silylating agent[103].

Table 11: Mass Spectra and Identities of Components in Blank Experiment Sample.

Peak No.	Identity	Certainty of Identity	Observed Mass Spectra (m/e, %)						Reference Mass Spectra
1	air	* * *	40	32	28	16	14		
			100	55	48	22	15		
2	carbon dioxide	* * *	44						168
			100						
3	acetone	* * *	43	58	28	42	27	29	168
			100	29	24	12	8	8	
4	trimethyl-silanol	* * *	75	45	47	76	77		168
			100	25	17	7	3		

*** - see Table 12

9c: GC/MS Analysis of an Aroma Isolate from Beef Cooked for One Hour

and

9d: GC/MS Analysis of an Aroma Isolate from Beef Cooked for Four Hours.

Mass spectra and identities for the aroma components isolated from beef cooked for 1h and 4h are presented in Tables 12 and 13 respectively and the relevant chromatograms shown in Figs.27 and 28(p.63,64). Although several components were identified in both samples, it was not possible to combine the Tables since the two sets of chromatograms could not be cross-correlated with absolute certainty. However it is convenient to discuss the two sets of results together.

A total of 78 identities were made and are summarised into chemical classes in Tables 14 and 15. Of these,12 have not previously been identified in cooked beef aroma. These are:

hept-3-ene*
a methylpentanolactone
a C_4 amine M=73
a methylfuroate
trimethylamine
3-isopentyl-2,5-dimethylpyrazine*
2,6-dimethyl-3-ethylpyridine*
2-isopropyl-4-methyl-5-ethylthiazole
2-propyl-4,5-dimethylthiazole*

chlorobenzene
tetrachloromethane*
1,1,1-trichloroethane

Of these, 5 (marked*) have not been identified in any food aroma.

Table 14: Classes and Numbers of Compounds Identified in Beef Aroma Samples.

Class of Compounds	1h cooking period	4h cooking period
aliphatic hydrocarbons	5	2
aliphatic alcohols	5	1
aliphatic aldehydes	6	5
aliphatic ketones	4	2
carboxylic acids	-	3
lactones	1	1
amines	1	1
benzenoid compounds	2	2
Cl-containing compounds	2	2
silicon compounds	5	4
aliphatic sulphur compounds	5	8
thiophens	-	2
furanoid compounds	-	9
pyridines	-	2
pyrazines	-	5
thiazoles	-	6
oxazolines	1	2

On a general level, the main differences between these samples are:

(i) an increase in the number of identifications made in the 4h sample. It is most likely that this is a direct consequence of longer cooking which has enabled production of higher concentrations of volatiles and thus facilitated their detection and/or identification. This effect is particularly the case for the heterocyclic compounds identified; in the 1h sample just one (excluding silicon compounds) was found, whereas 25 were identified in the 4h sample.

(ii) The number of aliphatic sulphur compounds identified increased in the 4h sample.

(iii) An increase in the number of carboxylic acids but decrease in the number of hydrocarbons and alcohols found in the 4h sample is also quite marked.

The formation of the compounds identified in the 2 beef aroma samples will now be discussed with reference to other reports of their identification in beef/model reactions.

Hydrocarbons are recognised principally as oxidative degradation products of fat[50], and thus relatively few have been found in either sample because the extramuscular fat was trimmed from the beef before cooking. Alkenes may also be formed by decarboxylation and deamination of aliphatic amino acids. Further reaction with hydrogen produces the corresponding alkanes[12]. Large numbers of hydrocarbons have been identified by workers heating beef fat or beef without the excess fat removed[5,17,48,139]. Lipids are thought to act as carriers of produced meat aromas[175], but are not responsible *per se* for the formation of characteristic meat aroma[176].

The lack of extramuscular fat in the beef used is also the reason for the small number of alcohols identified. This was also the experience of Min *et al.*[55]. Pentan-1-ol could arise from oxidation of linoleic acid[177]. Methanol and ethanol are thermal degradation products of glucose[178] and also of the Amadori compounds of glucose/glycine and of fructose/glycine[179]. Ethanol also forms from glucose/cysteine and glucose/cystine mixtures[180]. These lower alcohols, identified in the 1h sample, have previously been found by other workers in canned beef[47], and octan-1-ol by Min *et al.* in roast beef[55].

Acetaldehyde forms from pyrolysis of phenylalanine[181], of cysteine[182], of α-alanine[183] and of methionine[182]. Propanal and methylpropanal are the Strecker aldehydes of α-aminobutyric acid and of valine respectively and may also result from pyrolysis of methionine[182]. Propanal may also form from the degradation of the fructose/glycine Amadori product[179]. Acetaldehyde, propanal, methylpropanal, 2-methylbutanal and but-2-en-1-al are all thermal degradation products of glucose[184]. 2-Methylbutanal and 3-methylbutanal may also form from Strecker degradation of isoleucine and leucine respectively[185] and are pyrolysis products of amino acids[186]. But-2-en-1-al, hexan-1-al and propanal result from glucose/cysteine mixtures[187,188]. The aldehydes represented >42% of the 4h aroma sample. Liebich *et al.* found carbonyls to be the major part of roast beef aroma and especially of the roast beef drippings but these were probably the result of heating beef fat in air[27]. The production of 2- and 3-methylbutanal paralleled the development of flavour of roasted filberts[189]. Persson and von Sydow have identified methylpropanal as a major contributor to the burnt

Table 15: List of Compounds Identified in Beef Aroma.

Aliphatic Hydrocarbons
- pentane[a]
- hexane[b]
- heptane[a]
- 3-methylpentane[a]
- a hydrocarbon (poss. methyl propane)[a]
- a C7 hydrocarbon[b]
- hept-3-ene[a]

Aliphatic Alcohols
- methanol[a]
- ethanol[a]
- butan-1-ol[a]
- pentan-1-ol[a]
- octan-1-ol[a]
- dodecan-1-ol[b]

Aliphatic Aldehydes
- acetaldehyde[a,b]
- propanal[a,b]
- hexan-1-al[a]
- methylpropanal[a,b]
- 2-methylbutanal[b]
- 3-methylbutanal[a,b]
- but-2-en-1-al[a]

Aliphatic Ketones
- acetone[a]
- pentan-2-one[a]
- decan-2-one[b]
- butan-2,3-dione(diacetyl)[a]
- 3-hydroxybutanone[a,b]

Carboxylic Acids
- acetic acid[b]
- butanoic acid[b]
- hexanoic acid[b]

Lactones
- a methylpentanolactone[a,b]

Amines
- a C_4 amine, MW73[a]
- trimethylamine[b]

Benzenoid Compounds
- toluene[a]
- a benzenoid compound[b]
- benzaldehyde[a,b]

Cl-containing Compounds
- tetrachloromethane[b]
- 1,1,1-trichloroethane[a]
- chlorobenzene[a,b]

Silicon Compounds
- hexamethyldisiloxane[a,b]
- trimethylethoxysilane[a]
- trimethylsilanol[a,b]
- hexamethylcyclotrisiloxane[a,b]
- octamethylcyclotetrasiloxane[a,b]

Aliphatic Sulphur Compounds
- methanethiol[a]
- ethanethiol[b]
- carbonyl sulphide[b]
- dimethylsulphide[a,b]
- carbon disulphide[a,b]
- dimethyldisulphide[a,b]
- ethylmethyldisulphide[b]
- dimethyltrisulphide[a,b]

Thiophens
- a thiophen[b]
- a methylthiophen[b]

Furanoid Compounds
- furan[b]
- 2-methylfuran[b]
- 3-methylfuran[b]
- 2-ethylfuran[b]
- 2-n-pentylfuran[b]
- 2-furaldehyde(furfural)[b]
- 2-(hydroxymethyl)furan[b]
- a methylfuroate[b]
- 2-methyltetrahydrofuran-3-one[b]

Pyridines
- a methylpyridine[b]
- 2,6-dimethyl-3-ethylpyridine[b]

Pyrazines
- a dimethylpyrazine(2,5-or2,6-)[b]
- an ethylmethylpyrazine[b]
- a dimethylethylpyrazine[b]
- a C_5 substit.pyrazine(poss 2-ethyl-3,5,6 -trimethyl)[b]
- 3-isopentyl-2,5-dimethylpyrazine[b]

Thiazoles
- a thiazole[b]
- 2,4-dimethyl-5-ethylthiazole[b]
- 2-isopropyl-4-methyl-5-ethylthiazole[b]
- 2-propyl-4,5-dimethylthiazole[b]
- a C7 substit.alkylthiazole[b] or an acylthiazole
- benzothiazole[b]

Oxazolines
- 2,4-dimethyl-3-oxazoline[b]
- 2,4,5-trimethyl-3-oxazoline[a,b]

Miscellaneous
- carbon dioxide[a,b]
- Sulphur dioxide[b]

[a] - indicates presence in 1h aroma isolate
[b] - indicates presence in 4h aroma isolate

off-flavour of canned beef aroma[190].

Acetone, present due to the silylation procedure, may also result from pyrolysis of β-alanine[183], methionine[182], cysteine[191] and serine[192]. Butan-2,3-dione (diacetyl) is a reaction product of glucose/cysteine mixtures[182]. Pentan-2-one may result from thermal degradation of glucose[184]. 3-Hydroxybutanone (acetoin) is a result of degradation of the fructose/glycine Amadori product[179]. The relative absence of fat in this study makes the formation of aldehydes and ketones from Strecker degradation, Maillard browning and caramelisation reactions more likely[85,192,193]. Simple heat treatment of carbohydrates produces some α-dicarbonyls which react further with amino groups in Strecker degradations to form aldehydes. Hodge has suggested that 'browned', roasted, toasted notes arose from many Strecker aldehydes[192]. It has also been shown that the mode of cooking can affect the content and type of carbonyl aroma components in beef[142]. In Strecker degradation, a ketone is formed when the hydrogen on the α-carbon atom of the amino acid is substituted by an alkyl group[176]. The ketones found in these samples have all been previously identified in roast beef[27,55].

Acetic acid and butanoic acid both form from the thermal degradation of glucose[178,184]. Acetic acid also forms as a pyrolysis product of β-alanine[141,183], from glucose/glycine and fructose/glycine Amadori compounds[179], by Strecker degradation of cystine/pyruvaldehyde[180] and from glucose/cystine mixtures[180]. Amino acid pyrolysis studies and sugar degradation model reactions have been carried out at temperatures of 200°C and above[12], thus the presence of such reaction products at roasting temperatures is favoured. Carboxylic acids constitute < 1% of the 4h aroma sample. These acids have been found in beef broth[25] and boiled beef[23]. Tentative identification of a methylpentanolactone was made. Lactones are associated with thermal oxidation of lipids.

Several benzenoid compounds were identified. Degradation of benzenoid amino acids is probably the most likely formation route e.g. toluene from phenylalanine, benzaldehyde from phenylglycine. Toluene also forms from the degradation of glucose[184] and from glucose/cysteine mixtures[187]. Benzaldehyde may also arise from linoleic acid[194,195].

Chlorine-containing compounds have previously been found in heat-treated beef but none of the 3 identified in these samples, i.e. 1,1,1-trichloroethane, chlorobenzene and tetrachloromethane. It is most likely that these are contaminants, possibly deriving from pesticides[196]. Other contaminants are the silicon compounds. These arose from the heated glass flask or from the silylating agent. Unfortunately it was not possible to eliminate them without creation of many more contaminants derived from heated glass. Silylation replaces active hydrogen sites, thereby enhancing necessary stability of the system. However hydrolysis occurs at low temperatures in dilute solution and in nearly all reactions, in which the trimethylsilyl group is hydrolysed off an organic molecule, silanols result. In the presence of either base or acid, there is rapid formation of the corresponding siloxanes[197].

A total of 8 aliphatic sulphur compounds were found, comprising 2 thiols, 2 sulphides, 3 disulphides and a trisulphide- all of which have previously been identified in beef[28,47,138]. However, of these the following have not been cited in <u>roast</u> beef:- carbonyl sulphide, carbon disulphide, dimethyltrisulphide, methanethiol and ethanethiol. The formation of sulphur compounds in foods, comprehensively reviewed by Schutte[198] and Maga[159,199-201], involves thermal degradation of sulphur-containing amino acids and also the reaction of thermal degradation products of sugars with H_2S . The concentration of H_2S and other reactive sulphur compounds such as methanethiol and ethanethiol is known to increase with increased heating time[47,56]. Essentially, as the temperature reaches 50°C more thiol groups are produced by Strecker degradation and at 90°C these are oxidised to sulphides, disulphides and trisulphides releasing H_2S[10,12].

Both methanethiol and ethanethiol result from the Maillard reaction involving sulphur amino acids e.g. glucose/cystine[188], as well as cysteine/cystine-ribose mixtures[203]. Methanethiol is also a pyrolysis product of cystine[182] and of methionine[182] and it readily yields dimethylsulphide[10]. These compounds also form readily from decomposition of methional, the Strecker aldehyde of methionine[10,177]. Thiols also undergo equilibrium reactions with disulphides[204]. Carbon disulphide, carbonylsulphide and sulphur dioxide are

pyrolysis products of cysteine, cystine and methionine[141]. Carbon disulphide may also form from cysteine/cystine-ribose mixtures[203]. H_2S, which forms from all sulphur amino acids[182], is an important precursor of other sulphur-containing compounds e.g. by reacting with aldehydes to give dioxathianes, oxadithianes and trithianes[205], one of the latter class having been identified in cooked beef[28,206]. The reaction of H_2S with 4-hydroxy-5-methyl-3(2H)furanone is believed to be critical[11,201]. The aliphatic sulphur-containing compounds comprise > 53% of the total peak area in the 1h sample, and it is likely that the relatively smaller proportion identified in the 4h sample (25% of the total peak area) is due to their increased participation in secondary reactions during the longer cooking period e.g. methanethiol was not even identified in the 4h sample but comprised 11% of the total peak area in the 1h sample. Also the incorporation of sulphur into ring systems is favoured by increased heating periods[207]. This is confirmed in this study.

2-Methylthiophen may form as a result of pyrolysis of cysteine[208] and of thiamin [209,210]. 3-Methylthiophen results from Strecker degradation of cysteine and pyruvaldehyde[180]. Both methyl substituted thiophens are also products of cysteine Maillard reactions e.g. glucose/cysteine[187].

Trimethylamine has previously been found in steam distilled pork volatiles[211]. This may have been the result of decarboxylation of amino acids or may arise from lecithin. Enzymic conversion from trimethylamine oxide in fishery products has been established[212].

The 9 furanoid compounds identified have all been found in heat-treated beef[23,47,55]. Sugar/amine reactions[193], the action of fat as a precursor[47] and sugar degradation reactions[193] may all contribute to the formation of furans. Furan, 2-methylfuran, 3-methylfuran, 2-ethylfuran, 2-furaldehyde, 2-methyltetrahydrofuran-3-one and methylfuroate are thermal degradation products of glucose[184,213]. Both 2-furaldehyde and 2-(hydroxymethyl)furan form from glucose/cystine[180] and cysteine/cystine-ribose mixtures[203]. The 2-(hydroxymethyl) furan may also result from glucose/glycine and fructose/glycine Amadori compounds[179]. Both 2-methylfuran and 2-methyltetrahydrofuran-3-one are thermal degradation products of thiamin[210]. The 2-n-pentylfuran may derive from oxidised linoleic acid[214].

The absence of furanoids in the 1h sample probably resulted from the relatively short time for which the meat reached roasting temperatures. Furanoid compounds represent about 3% of the total peak area in the 4h sample.

The two pyridines identified may have been formed by reaction of aldehyde and NH_3 followed by aldol-type condensation of the newly formed imine[49,215]. Pyridines are also produced by the pyrolysis of amino acids e.g. cystine,[208] β-alanine,[183] as well as by the Maillard reaction e.g. glucose/cystine and glucose/cysteine [180,187]. The reaction of proline and glucose under roasting conditions also produces pyridines[216]. 2,6-Dimethyl-3-ethylpyridine has not previously been identified as a beef aroma volatile nor in any other food aroma[217]. However methylpyridines have previously been found in heated beef[4,5]. In the 4h sample the two pyridines constitute < 1% of the total peak area. Five pyrazines were identified and all but 3-isopentyl-2,5-dimethylpyrazine have been reported in heat-treated beef[29,30,50,153]. The formation of this class of compounds has been extensively studied[218-221]. Concluding from these papers, pyrazines are formed from sugar-amino condensation reactions which are affected by factors such as temperature, time, reactant ratio and presence of acid or base. Koehler and Odell determined by C_{14}-labelling that the sugars provided the carbon atoms in pyrazines, and amino acids (and not NH_3) the nitrogen[222]. Pyrazines have also been shown, in model systems to form by cyclisation of hydroxy-amino acids e.g. threonine and serine[223].

Sugar/NH_3 model systems give a wide variety of pyrazines which depends on the ∝- amino carbonyl intermediates formed[219]. Several pyrazines were reported by Shibamoto and Russell among the reaction products of glucose/NH_3 and H_2S[224]. Wasserman suggests that when meat is first heated (pH 5.5) pyrazine formation by the carbonyl/amino acid mechanism is probably favoured but as heating continues and more NH_3 is liberated, the increased pH is likely to favour the reaction described by Shibamoto and Russell[225].

Although no pyrazines were identified in the 1h sample they represented < 1% of the total peak area in the 4h sample.

Six thiazoles were identified in the 4h aroma sample. They may form from thermal degradation of cystine[208], of

thiamin [210] and by Strecker degradation of cysteine/cystine-pyruvaldehyde mixtures[180]. Also formation by the Maillard reaction between glucose and cystine may be involved[180]. In particular, 2,4-dimethyl-5-ethylthiazole is a reaction product of cysteine/cystine-ribose.[203] It has been suggested by Mulders that the sulphur and nitrogen atoms are contributed from cysteine and the side chains by α-dicarbonyls which are sugar degradation products[203]. Reaction involving α-dicarbonyls and aldehydes, NH_3 and H_2S form thiazoles as well as other sulphur heterocycles[205]. Suggested formation pathways have been summarised by MacLeod and Seyyedain-Ardebili[13]. Of the thiazoles identified, neither 2-isopropyl-4-methyl-5-ethylthiazole nor 2-propyl-4,5-dimethylthiazole has been identified in beef aroma and the latter not in any food aroma[217].

Both oxazolines found have previously been identified in beef[23,137,148]. Mussinan et al. found thiazolines to be less stable to heat than the corresponding oxazolines[137]. This may account for the absence of thiazolines in these samples, since oxidation of thiazolines yields the corresponding thiazoles[226].

The reaction between acetaldehyde, NH_3 and 3-hydroxybutanone (acetoin) produces 2,4,5-trimethyl-3-oxazoline[137]. Both acetaldehyde and 3-hydroxybutanone were identified in these beef aromas. Another possible formation pathway for this oxazoline is by the reaction of alanine and butan-2,3-dione[227]. The 3-oxazolines represent < 2% of the total peak area in the 4h sample.

Schwimmer and Friedman concluded that in a harsh treatment such as roasting, more of the sulphur present in the system is taken into heterocyclic ring compounds[207].

This agrees with the present findings in that the harsher treatment of the 4h sample produced many more sulphur-containing heterocycles than the 1h sample.

The relevance of the heterocycles to cooked beef aroma has been stressed by many workers[50,228]. Recently Ching pointed out that both heating time and temperature were critical to meat aroma development[138]. Thiophens and pyridines have also been found in meat heated for longer periods to higher temperatures[126]. Koehler and Odell found pyrazine

yield at 120°C to increase with time up to 72h[221]. Satyanarayan and Parihar stated that the main role of the heterocycles and especially the pyrazines, is to contribute to the aroma of roasted foods[229]. The near exclusive presence of pyrazines and thiophens in roasted/toasted foods indicates the contribution these classes make to such aromas.

It is gratifying to have obtained 25 identifications of heterocycles in the 4h sample. This is a marked increase over the previously MLC-sponsored project in which 17 heterocyclic compounds, but none with > 1 heteroatom, were identified [2,5]. Eight multi-heteroatomic compounds were identified in the $^{4h}\lambda$ aroma sample. The high number of aliphatic hydrocarbons (28) identified in the previous project may have been due to inadequate removal of extramuscular fat. There has also been a very large increase in detection of sulphur compounds, both aliphatic and heterocyclic, in the present project (19 as opposed to 3 previously). Herz and Chang emphasised the direct contribution of aliphatic sulphur compounds and furanoids (8 identified in this study) to cooked beef aromas[228].

The challenge to identify key components possessing characteristic aromas has led to the identification of many possible candidates, all of which have been heterocyclic e.g. 2,4,5-trimethyl-3-oxazoline[148], 3,5-dimethyl-1,2,4-trithiolane[148], 5,6-dihydro-2,4,6-trimethyl-1,3,5-dithiazine (thialdine)[52], 4-hydroxy-5-methyl-3 (2H)-furanone and its 2,5-dimethyl homologue[25]. Despite the many valuable, but as yet not totally successful, attempts to identify the key components of cooked beef aroma, numerous characteristic and powerful odourants, in trace quantities, remain to be identified[153].

PART II: THE SENSORY PROPERTIES OF COOKED BEEF AROMA.

INTRODUCTION.

Aroma is considered to be primarily a psychophysical concept and secondarily a chemical concept[230]. Von Sydow stated that the chemical origin of the perceived aroma of a food is the presence of one or several chemicals in the food, and that equal emphasis was required for instrumental analysis of volatiles and sensory analysis of perceived aromas, both of which should preferably be performed in parallel[231].

Whereas instrumental techniques are objective, sensory analysis necessitates evaluations by humans and may therefore be subjective. In an excellent paper, Köster discussed the internal and external factors affecting sensory analysis, their interdependence and ways in which their influence on measurements by humans might be reduced[232]. He also pointed out the tendency to forget the limitations of humans whose assessments involve sensitivity, reliability, linearity and systematic error. Additional to these factors are those which affect these human limitations and have been classified as psychophysical, physiological and environmental[233].

In light of the view of Seidman that "odour is probably the most complicated of our sensations because of the multitude and variety of smell stimuli we encounter in our daily life"[234], the influencing factors and limitations described above are of secondary importance when the lack of understanding of the mechanisms and processes involved in aroma perception are considered[235].

There are many different methods for sensory analysis, their use being dependent on the type of information sought. Von Sydow pointed out that sensory analysis be sufficiently detailed and sensitive to distinguish quantitative and qualitative changes and discrepancies in samples[231]. The choice and limitations of sensory methods have been well summarised by Larmond[236]. Methods include difference tests, sensitivity tests, preference tests and descriptive tests. It is to the latter that attention will be focussed because they enable a detailed analysis of complex aromas, unlike the other comparatively simple tests mentioned.

Descriptive analysis originated as The Flavour Profile Technique, introduced by A.D. Little Inc. Cairncross and Sjöstrom defined flavour profile as 'expressing in common language terms the characteristic notes of both aroma and flavour, their order of appearance and intensities and the amplitude of total aroma and flavour[237]. At best, the technique was only semi-quantitative and has been little used since the 1950's. However certain subsequent improvements have allowed the flavour profile concept to be modified and to be quantitative i.e. by combining descriptive tests with category or ratio scaling[238]. For example, assessors are trained to perceive, describe and quantify particular aroma attributes having first agreed on a set of standards. Experience and training of assessors increase the objectivity of these tests. The advantages of such techniques are that the testing of aromas requires no memory of, or reference to, a particular sample such that differences can be detected from the descriptions obtained[239]. Some selection and training however is advised[239].

By virtue of the subjectivity of descriptive terms used in this type of sensory analysis, many workers stressed the need for standardised terminology[240,241], and have compiled aroma vocabularies for particular foods thereby increasing relevancy of the descriptors to the food being analysed[239,242-244]. The number of odour terms used can be quite high, e.g. Harper et al.used assessors to characterise 53 odour stimuli and from those chose 44 adjectives to serve as odour qualities(OQ's)[245]. An increased number of variables leads to higher possibilities of co-linearity i.e. interdependence of variables[246]. Decreasing the number of sensory terms has been achieved on the basis of frequency of use, in describing particular aromas[3,247,248], and/or by statistical means i.e. factor analysis.[247,249-251].

Factor analysis is a multivariate method which aims to achieve an orderly simplification of a number of interrelated measures. It is based on the faith that the observed correlated variables are mainly the result of some underlying regularity in the data, which can be explained in terms of a few conceptually-meaningful, relatively independent factors. Thus when a group of variables has a great deal in common, a factor may be said to exist and it is the primary task of factor analysis to extract such factors. These factors may be used as independent or dependent variables in later analyses e.g. regression and

discriminant analysis[252]. The use of factor analysis, as a data-reduction tool, has achieved considerable simplification of sensory aroma descriptors and thereby eased subsequent interpretation of results. Wu et al.,in a search for the main variables reflecting differences between red and white wines, applied factor analysis successfully to the 27 descriptors used and extracted 8 and 6 factors respectively[250].

In most cases, sensory analysis is performed on the original food sample, since sensory analysis of aroma isolates presents practical difficulties with respect to presentation to assessors. Odour port assessment (OPA) of the GC effluent, termed a form of qualitative analysis by Powers[235], is widely used although Tucknott and Williams have outlined the disadvantages of the technique[253]. These include the elevated temperature during assessment, the restriction to one assesor at a time without the aid of a multi-splitting device, the presence of components in concentrations greater than in nature and the necessity for rapid decisions for the assignment of descriptors, which may lead to poor or even false interpretation. Further to this, collecting the volatiles in cooled traps is often elaborate but the authors described a technique whereby syringes collect 10 - 20cm^3 aliquots for subsequent assessment. Other methods have involved tasting the GC effluents as aqueous solutions[254], collecting the effluent in a solid CO_2-cooled tube followed by centrifugation[255], and collecting in glass porous-layer open tubular capillaries which are crushed in ice-cold water and stored in vials for sniffing[98,256,257]. Dravnieks et al. recently discussed the variation induced by effluent sampling e.g. condensation or strong adsorption[258]. To minimise these (variations), all tubing leading out of the GC required heating such that the split ratio is not altered by temperature differences in the splitter branches. However the main disadvantage of OPA is that components are individually assessed such that effects of masking, synergism etc. on the combined aroma components cannot be estimated. Despite this, Noble stressed the necessity for OPA as a means of detecting components which do not form peaks but are important with respect to odour[259]. A search of the literature has produced few citations describing techniques whereby the 'total' aroma of an isolate may be qualitatively and quantitatively assessed[257].

Only two detailed and systemative descriptive analyses of cooked beef aroma have been performed. Notable work on the

retort flavour of canned beef stew by Persson et al. involved assessment by nose and mouth by trained assessors[260]. Other work by this group involved OPA only[47]. The second detailed analysis, carried out in these laboratories, has already been described on p. 7 [3]. In both studies sensory analysis was performed on beef samples and not beef aroma isolates.

Choice of Methods Used in this Project.

An aroma profile technique was developed to obtain sensory descriptions of the beef aroma isolates. Factor analysis was selected to reduce the number of sensory descriptors to a more manageable level. The advantages of this, besides the data reduction facility, was the likely decrease in colinearity of variables (which could later confuse interpretation of results) and decrease fatigue of the assessors by keeping descriptors to a minimum.

OPA was used to obtain sensory descriptions of the individual components eluting from the GC column, thereby allowing areas of sensory interest on the chromatogram to be pin-pointed. However to overcome the drawback of sequential assessment of individual components, a method was developed to enable presentation of the 'total' aroma of an aroma isolate for descriptive analysis by the assessors.

EXPERIMENTAL

EXPERIMENT 10: Factor Analysis of Previously Published Data[3].

The data of MacLeod and Coppock were used[1,3]. They had originally compiled a preliminary list of relevant OQ's from the vocabularies of Harper et al.[245,261] and Persson et al [260]. Subsequently 15 trained assessors had agreed on a revised list of 41 OQ's (see Table 16), each of which was scored for 16 cooked beef samples (boiled and roasted) on a linear 0-9 point category scale of intensity.

Factor analysis (FA) was applied to these data to search for similarities between samples so as to explain total cooked beef aroma, by performing an orderly simplification of the OQ's into groups of strongly related qualities or factors. Factor analyses were attempted using the scores given by individual assessors (rather than total panel scores) for 2 subsets of the data - (i) OQ's remaining after deleting those detected by less than half the assessors (see Table 16), since it was assumed that these did not contribute significantly to

Table 16: Odour Qualities Scored for Cooked Beef Aromas[1,3]

almond-like[a]
ammonia-like
animal/goaty
aromatic[b]
blood-like
broth-like
Bovril-like[b]
burnt
buttery
cool/cooling
cooked cabbage
cured meat/bacon[a]
earthy/soil
flat/dull
fragrant
garlic/onion[a]
herbal/dried herbs/hay
irritating on nose[a]
Marmite-like[b]
meaty-boiled
meaty-roast
meaty-raw
musty/mouldy
metallic[a]
nasty smelling[b]
oily/fatty
paint-like
rancid[a]
rubber/burnt[a]
sausage-like[a]
savoury[b]
sickly[b]
sharp/pungent
sweaty
sweet
spicy
sulphurous[a]
throaty[b]
toasted
vegetables overcooked
yeasty[a]

Also scored were {overall odour strength and preference}

Footnote: [a] indicates those odour qualities detected by < ½ the panel of trained assessors and usually at low intensity levels.

[b] indicates qualities of a relatively subjective nature or else not very useful as true objective descriptors.

sensory description of cooked beef aroma, (ii) OQ's remaining after deleting those of a relatively subjective nature (see Table 16), as well as those described in (i).

Factor analysis was performed using the University of California BMDP4M Computer Program[262]. Initial factor extraction was by principal components analysis followed by varimax rotation (orthogonal) in which the principal axes were rotated about the origin whilst maintaining the axes at 90°.

EXPERIMENT 11: Validation of the Factor Analysis Results of Expt. 10.

Seventy five untrained assessors were presented with 180g samples of variously heated beef devoid of extramuscular fat i.e. raw, boiled (1kg/1h) and roasted (1kg/1h/205°C), and a record form listing the 24 OQ's used in the FA described in Expt. 10(ii). Assessors were requested to indicate the presence, if perceived, of each OQ in each of the 3 aromas. Samples were presented at room temperature in masked bottles with screw lids.

EXPERIMENT 12: Sensory Analysis of Cooked Beef Aroma Isolate (the GC Technique).

Using the technique described in Expt. 5, the perceived aroma of an isolate obtained as in Expt. 4b was described by four experienced female assessors (a) using the odour factors resulting from the FA in Expt. 10(ii), - see Table 18 , and (b) using the 24 objective OQ's used for the FA of Expt. 10(ii) - see Table 16 . The assessors were requested to score each descriptor on a linear scale of 0 - 5 where 0 represented absent and 5 respresented strong. An intensity rating was required for each of the three stages of the heat desorption procedure, namely the N_2 pre-flush period, the heating period and the flush-on period.

EXPERIMENT 13: Development of a Mixing Chamber to Improve the Sensory Analysis of the Total Aroma Isolate (the Globe Technique)

A technique was developed to enable sensory analysis of a total aroma isolate by a panel of assessors and to overcome the partitioning effect evidenced in Expt. 12. This essentially involved desorption of a sampled Tenax tube into a partially-evacuated glass globe which had 4 odour ports positioned around its horizontal circumference i.e. the globe acted as a mixing chamber.

Fig.29: The Globe Mixing Chamber Apparatus used for Sensory Analysis - Expt. 13(i)

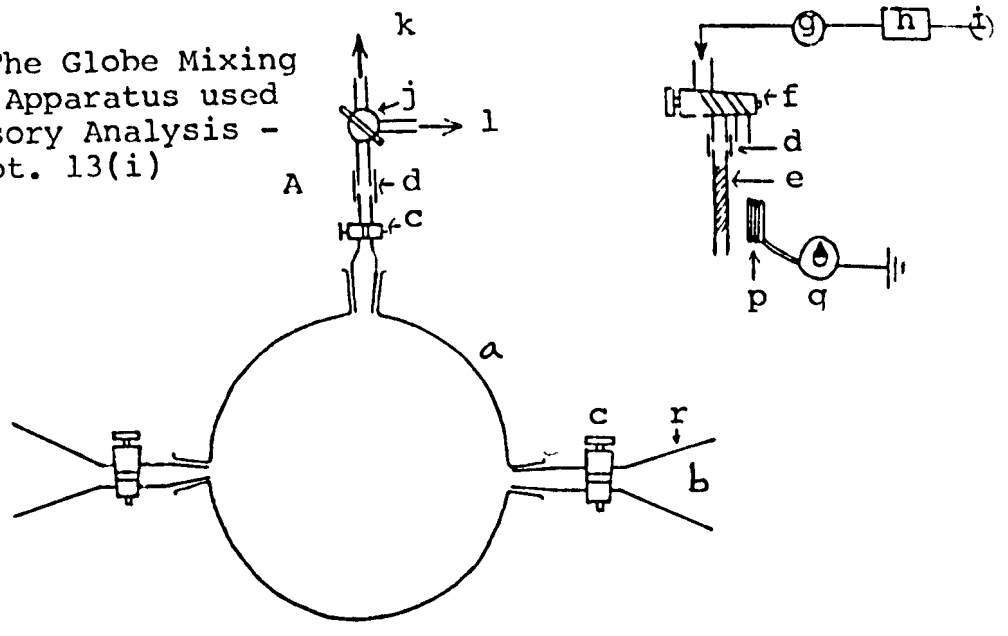
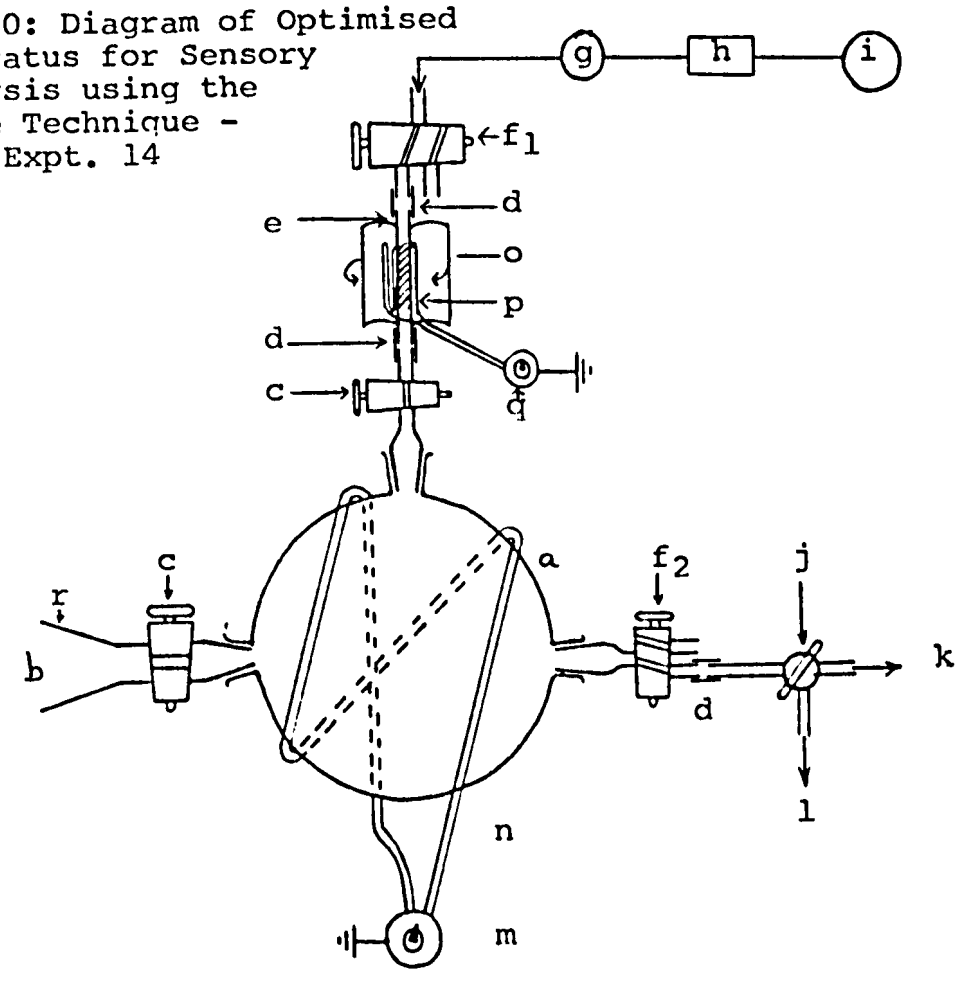


Fig.30: Diagram of Optimised Apparatus for Sensory Analysis using the Globe Technique - Expt. 14



Key:

a. 21. glass flask
b. Odour port(one of 4)
c. On/off tap
d. Polythene tubing
e. Sampled Tenax tube
f. Double oblique glass tap
g. Flowmeter
h. Molecular sieve 13X
i. N_2 cylinder
j. 3/way tap
k. to vacuum pump
l. to atmosphere
m. Variac controller
n. Heating coil(0.6m)
o. Heater insulation
p. Heater
q. Energy regulator
r. Glass funnel

flushed into the globe for 15 min. By monitoring the N_2 flow constantly and by suitable use of the tap at f_1 it was possible to maintain the pressure within the globe at or near atmosphere. Four experienced assessors sniffed at the odour ports and described the perceived aroma using the lists of factors and OQ's as described in Expt. 12.

EXPERIMENT 15: Gas Chromatographic Odour Port Assessment of Individual Aroma Compounds.

The odour qualities of the individual separated GC peaks were assessed on elution from a 5.5m x 0.6mm o.d. PEG 20M column connected to a silylated splitter (ratio 10:1 to the detector). The odour port heater was set at 225°C. All other GC conditions were as described in Expts. 1b and 2c. Four untrained assessors who were experienced in sensory analysis sniffed and described, in their own words, the aromas desorbed from a Tenax tube, sampled as in Expt. 4b, as they eluted at the GC odour port. Each assessor sniffed for four 20 min. sessions and the descriptions recorded on the appropriate chromatographic peaks.

EXPERIMENT 16: Fractionation of 'Total' Aroma Sample and Sensory Analysis of Fractions using
(a) The GC Technique, and
(b) The Globe Technique.

From the OPA in Expt. 15 it was noted that many of the "meaty" terms coincided with small peaks, valleys between peaks or shoulders of large peaks. It was therefore attempted to fractionate out the smaller peaks into one Tenax tube and the remaining larger peaks onto another receiver tube, with the intention of obtaining aroma descriptions for the simplified fractions. The division of fractions is demonstrated in Fig.31, p.124.

Collection of the two fractions onto receiver tubes was performed as described in Expt. 7. The two Sba receiver tubes were interchanged repeatedly at the GC column exit until the total aroma isolate obtained as in Expt. 4b, had been fractionated. The solid CO_2 was replenished throughout. Sensory analysis by 4 assessors by the GC and Globe Techniques was carried out using the aroma profile techniques described in Expts. 12 and 14. GC monitor chromatograms of the aroma fractions were also obtained using the GC conditions of Expt. 1b as modified in Expt. 2c.

RESULTS AND DISCUSSION

EXPERIMENT 10: Factor Analysis of Previously Published Data[3]

Rotation attempts to redistribute the experimental variance amongst the extracted factors, and by rotating orthogonally the resultant derived factors are not intercorrelated. (i) Using sensory scoring data of 33 OQ's (having deleted those scored by < half the assessors) the unrestricted factor analysis (FA) extracted 10 factors explaining 67.6% of the total variance in the aroma of the 16 cooked beef samples. The communality estimate for each of the 33 OQ's was > 60% in the case of all but 3 OQ's (i.e. sweaty at 58.6%, sweet at 43.2% and flat/dull at 55.2%). This meant that over 60% of the variability of 30 out of 33 OQ's analysed was explained by the 10 factors extracted. The results of this FA are shown in Table 17 such that only those OQ's with factor loadings $\geq \pm 0.25$ are included. Factor loadings are the equivalent of correlation coefficients for FA. They represent the degree of association of an explanatory variable to that factor such that variables with high factor loadings exert greater influence on the factor than those of low loadings or less close association.

Values $\geq \pm 0.45$ were considered to represent strong association between an OQ and a factor. Frequently, loadings of $\geq \pm 0.3$ are taken as an arbitrary criterion of significance although Child recommended $\geq \pm 0.483$ at the 5% level of significance (and $\geq \pm 0.605$ at the 1%) for a sample size of 16 [263]. However the patterns of insignificant loadings in Table 17 substantiate the interpretation of the significant loadings[263]. Any OQ loading onto > 1 factor, said to have a complexity > 1, becomes difficult to interpret simply, as it measures more than one theoretical dimension.

Overall it can be concluded that Factors 1, 2 and 3 describe meaty raw, boiled and roast properties. They indicate those OQ's which are strongly associated with these meaty aromas of the variously heated beef samples analysed. In particular, F1 represents relatively undesirable beef aroma qualities; F2 describes a high intensity of desirable meaty roast characteristics; F3 does not discriminate effectively between specific aromas produced by different cooking methods.

Factors 4 to 10 inclusive highlight specific qualities

of the cooked beef aromas, which generally, are not included in Factors 1 to 3. However F4 and F7 contain odour qualities showing close associations with meaty aromas i.e. meaty-boiled-associated qualities in F4 and meaty-roast-associated qualities in particular in F7. An interesting bipolar relationship of "meaty boiled" vs. "meaty roast" is represented by F7.

Factors 1 to 4 and 7 indicate the following division of odour qualities most strongly related to boiled beef aroma and roast beef aroma, respectively, in the 16 samples analysed.

meaty raw/boiled	meaty roast
sickly F1	savoury F2
blood-like F1	Bovril-like F2
nasty smelling F1	(meaty-roast F3)
earthy, soil F1	burnt F7
sweaty F1	toasted F7
animal, goaty F1	
cool, cooling F1, F4	
oily, fatty F1	
musty, mouldy F1	
meaty-raw F1, F3	
meaty-boiled F1, F3	
broth-like F3	
Marmite-like F3	
vegetables, overcooked F4	
cooked cabbage F4	

F1, F3, F4 represent qualities associated with meaty-raw/meaty-boiled aromas. F2, F7 represent qualities associated with meaty-roast aromas.

In addition to general meaty qualities and qualities which discriminate most effectively between boiled and roasted aromas of heated beef, the cooked beef aromas analysed also possessed a certain pungency F5, fragrance F6, aromatic qualities F8, paint-like qualities F9 and ammoniacal qualities F10. The 10 factors may therefore be interpreted as:

F1: The General Factor
The Meaty Boiled Factor

F2: The Meaty Roast Factor

F3: The Meaty Factor

F4: The Cooked Vegetables Factor

F5: The Pungent Factor

F6: The Fragrant Factor
F7: The Burnt Factor
F8: The Aromatic Factor
F9: The Paint-like Factor
F10: The Ammoniacal Factor

(ii) An unrestricted FA on the 24 most objective OQ's extracted 9 factors which explained 69.4% of the total variance in the aromas of the 16 cooked beef samples. Of these, the first 6 each accounted for > 5% of the total variance. Only 2 of the OQ's, meaty-roast and meaty-raw, loaded significantly onto more than one factor. The results of this FA are presented in Table 18 . In comparison with Table 17 it is immediately obvious that a 'cleaner' solution was obtained in that fewer OQ's had low factor loadings. Also fewer OQ's had a complexity >1. Thus the factors were more divisive and more amenable to interpretation.

Factor 1 is the general factor containing meaty terms and characterised by broth-like, meaty-boiled, meaty-roast and meaty-raw. It does not discriminate between specific aromas but denotes a general meatiness. F1 explains 15.8% of the total variance.

Factor 2 is a more specific factor strongly associated with overcooked vegetables, cooked cabbage and cool, cooling qualities. Less closely associated are the OQ's blood-like, sweaty and meaty-boiled. Thus a slight meaty-boiled character is present with the cooked vegetable qualities. F2 explains 10.7% of the total variance.

Factor 3 is characterised by highly significant loadings for toasted, burnt and meaty roast. This specific factor clearly discriminates the roasted/toasted character of beef aromas. F3 accounts for 8.7% of the total variance.

Factor 4 is strongly associated with OQ's ammonia-like, animal/goaty, sharp/pungent and earthy/soil. F4 explains 7.8% of the total variance.

Factor 5 is defined by the OQ's oily/fatty and paint-like. There are less close associations with earthy/soil, blood-like and cool, cooling. It represents 6.9% of the total variance.

Factor 6 is characterised by strong associations with spicy, fragrant, sweaty and to a lesser extent herbal/hay. F6 accounts for 5.5% of the total variance.

Factor 7 is defined by musty/mouldy. Weakly associated OQ's

are animal/goaty, sweaty and blood-like. F7 explains 4.9% of the total variance.

Factor 8 is strongly characterised by flat/dull, meaty-raw and herbal/hay and accounts for 4.7% of the total variance.

Factor 9 is defined by buttery and a very weak association with sweet. F9 explains 4.4% of the total variance.

Overall this FA can be classified as:-

Factor 1: The Meaty Factor - a general factor of all 3 meaty OQ's

Factor 2: Cooked Vegetables Factor - a specific factor with meaty-boiled character.

Factor 3: Toasted/Burnt Factor - a specific factor of roast meat qualities.

Factor 4: Ammoniacal Factor - a specific factor with pungent characteristics.

Factor 5: Oily/Fatty Factor - a specific factor with paint-like character

Factor 6: Fragrant Factor - a specific factor of spicy/fragrant qualities.

Factor 7: Musty/Mouldy Factor - a specific factor

Factor 8: Flat/Dull Factor - a specific factor with meaty-raw character.

Factor 9: Buttery Factor - a specific factor.

Thus an uncomplicated list of a limited number of objective aroma qualities suitable for the descriptive analysis of cooked beef aroma isolates, and one which distinguished sensorially between boiled and roasted aromas, was obtained.

EXPERIMENT 11: Validation of the Factor Analysis Results of Expt. 10.

The results of the sensory analysis of the aromas of 3 beef samples (raw, boiled and roasted) by 75 untrained assessors are summarised in Table 19 . Frequency of use totals of > 18 were used, representing 25% of the total assessments.

Table 19 : Results of Factor Analysis Validation Experiment - Odour Qualities Particularly Associated with Different Beef Aromas and Their Frequency of Use.

Beef Aroma Sample	Odour Quality	Frequency of Use	Presence of OQ in Extracted Factor (Table 18)
Raw	animal/goaty	29	4
	blood-like	50	1
	flat/dull	21	8
	meaty-raw		1,8
Boiled	broth-like	44	1
	o/cooked vegetable	28	2
	cooked cabbage	23	2
	flat/dull	20	8
	meaty-boiled	52	1,2
	oily/fatty	19	5
Roast	burnt	48	3
	fragrant	29	6
	meaty-roast	67	1
	oily/fatty	30	5
	spicy	21	6
	toasted	25	3

The presence of these particular OQ's in the appropriate 9 factors of Table 18 is summarised:-

Raw beef aroma - Factors 1, 4, 8

Boiled beef aroma - Factors 1, 2, 5, 8

Roast beef aroma - Factors 1, 3, 5, 6

Both raw and boiled beef aromas, which were scored highly for flat/dull, are associated with Factor 8 - the Flat/Dull Factor. Likewise boiled and roast beef aromas are associated with Factor 5 the Oily/Fatty Factor. Clearly the FA has been able to discriminate between the 3 different beef aromas. Thus the 9 factors extracted from a FA on previously published data from trained assessors can be used by untrained assessors to describe beef aromas. The factor analysis was therefore validated. It was decided to use these 9 factors and the 24 OQ's as descriptors in the aroma profiling technique for the sensory analysis to follow.

EXPERIMENT 12: Sensory Analysis of Cooked Beef Aroma Isolate (the GC Technique)

Tables 20A and B show the intensity scores for odour factors and OQ's respectively from the sensory analysis of a total aroma isolate at the GC odour port.

Table 20A. Odour Factor Intensity Scores for Total Aroma Isolate (GC Technique) Expt. 12.

Odour Factor (Expt. 10(ii))		Intensity Ratings: N_2 Pre-flush period (4 assessors)	Heating Period (4 assessors)	'Flush-on' period (4 assessors)
meaty	F1	2,4,5,4	0,2,3,0	4,2,4,5
overcooked veg	F2	1,2,1,0	0,0,0,0	0,2,2,4
toasted/burnt	F3	0,1,2,0	0,0,0,0	2,2,3,5
ammonia-like/ pungent	F4	0,3,2,0,	0,0,0,0	2,4,0,0
oily/fatty	F5	0,0,4,2	0,0,1,0	0,0,2,0
spicy/ fragrant	F6	0,1,1,0	0,0,0,0	0,2,2,2
musty/mouldy	F7	0,0,0,2	0,0,1,0	4,0,0,0
flat/dull	F8	0,0,0,0	0,1,0,0	1,0,0,0
buttery	F9	2,2,5,5,	2,0,1,0	0,0,1,0

Table 20B: Odour Quality Intensity Scores for Total Aroma Isolate (GC Technique) Expt. 12.

OQ	Intensity Ratings: N_2 Pre-flush period (4 assessors)	Heating period (4 assessors)	'Flush-on' period (4 assessors)
meaty raw	0,0,0,0	0,0,0,0	1,2,0,0
meaty boiled	0,1,5,4	0,0,0,0	2,2,3,?
meaty roast	2,4,1,0	0,2,0,0	4,1,5,4
ammonia-like	0,0,0,0	0,0,0,0	2,0,0,0
animal/goaty	0,0,0,2	0,0,0,0	0,0,0,1
bloodlike	0,0,0,0	0,0,0,0	0,2,0,0
broth-like	0,1,3,4	0,0,0,0	1,1,0,0
burnt	0,1,0,0	0,0,0,0	3,2,3,5
cooked cabbage	0,4,0,0	0,1,0,0	0,2,0,3
vegetables overcooked	1,2,1,0	0,1,0,0	0,2,1,3
cool/cooling	0,0,0,0	0,0,0,0	0,0,0,0
earthy/soil-like	0,0,0,0	0,0,0,0	0,1,5,2
flat/dull	0,0,0,0	0,1,0,0	1,0,0,0
fragrant	0,0,0,0	0,0,0,0	0,0,2,2
herbal/hay	0,0,0,0	1,0,0,0	0,0,1,0
musty/mouldy	0,0,0,2	0,0,0,0	4,0,0,0
oily/fatty	0,0,4,2	0,1,0,0	0,0,2,0
paint-like	0,0,0,0	0,0,0,0	0,0,0,0
sharp/pungent	0,3,0,0	0,0,0,0	0,4,0,2
spicy	0,1,0,0	0,0,0,0	0,2,0,2
sweaty	0,0,0,0	0,2,0,0	0,0,0,3
sweet	0,0,5,5	0,0,0,0	0,0,3,1
toasted	0,1,0,0	0,0,0,0	3,0,4,5

The results, summarised below in Table C, show differences between the heat desorption periods as scored by the individual assessors. Factor numbers (from Expt. 10(ii)) are included in brackets.

Table 20c: Summarised Results of Sensory Analysis of Cooked Beef Aroma Isolate.

	Factor	Odour Quality
Higher Scores in N_2 Pre-flush period	buttery (F9)	meaty-boiled (F2) (F1)
	oily/fatty (F5)	sweet (F2)
		broth-like (F1)
		buttery (F9)
		ily/fatty (F5)
Higher Scores in Flush-on period	spicy/fragrant (F6)	fragrant (F6)
	NH_3-like/pungent (F4)	meaty-roast (F3) (F1)
	toasted/burnt (F3)	burnt (F3)
		earthy/soil (F4)
		spicy (F6)
		toasted (F3)
		sharp/pungent (F4)
Equally Scored in both periods	flat/dull (F8)	flat/dull (F8)
	musty/mouldy	animal/goaty (F8)
	meaty (F1)	herbal/hay (F8)
	o'cooked vegetables (F2)	musty/mouldy (F7)
		meaty/raw (F8)
		paint-like (F5)
		sweaty (F6)
		cool, cooling (F2)
		cooked cabbage (F2)
		blood-like (F1)
		o'cooked vegetables (F2)
		NH_3-like (F4)

It is interesting to note that factors containing the OQ's scored more highly in the N_2 pre-flush and flush-on periods respectively are mutually exclusive, excepting the general factor F1 containing all the meaty OQ's. Thus Factors 1 (meaty), 2 (cooked vegetables), 5 (oily/fatty), 9 (buttery) dominate the N_2 pre-flush period whereas Factors 1 (meaty), 3 (toasted/burnt), 4 (ammoniacal), 6 (fragrant) exclusively represent the flush-on period.

Thus it is clear that sensory differences are evidently due to a partitioning effect caused by the heat desorption procedure. This is easily explained on the basis that the N_2 pre-flush volatiles are desorbed by the ambient N_2 flow. Their aroma will predominate in this period whereas those components requiring heat for desorption will constitute the aroma perceived during the flush-on stage. Very little aroma was detected during the heating stage of desorption, when no N_2 flowed.

Despite the good detailed aroma descriptions so far obtained, the partitioning effect made it impossible to achieve an integrated description of a 'total' aroma sample. It was thus desirable to develop a method by which the 'total' aroma sample could be sniffed and described as an entity.

EXPERIMENT 13: Development of a Mixing Chamber to Improve the Sensory Analysis of the 'Total' Aroma Isolate (the Globe Technique).

(i) The descriptions of the aroma sample sniffed using the apparatus shown in Fig.29, p.89 were:- caramel, fatty, toffee, falt/dull, diacetyl and buttery. It was not very meaty and not meaty-roast. Although the globe technique appeared promising, roasted notes were not well perceived. This was probably due to excessive condensation and/or adsorption onto the large surface area of the ambient glass globe walls. It had been shown in Expts. 5 and 12 that roast qualities were preceived at the GC odour port when attached to a heated (175°C) empty glass column. Thus it was concluded that heating the globe was vital for good perception of typical roast beef qualities. It was also decided to silylate the glass globe.

(ii) The aroma descriptions obtained using the heated and silylated 2ℓ. globe with suitably modified evacuation and flush-on times were:- meaty, burnt, oily, fatty, charred, pungent, slightly boiled, burnt, roast and charcoaled steak.

It was concluded that qualitative transferral of volatiles desorbed from the Tenax tube to the 2ℓ. globe was being closely approached. A typical roast beef aroma was perceived at the globe odour ports.

EXPERIMENT 14: Sensory Analysis of the 'Total' Aroma Isolate (the Globe Technique)

Tables 21 A and B show the results of the sensory analysis, using the 2ℓ. globe depicted in Fig. 30 p.89 of a 'total' aroma isolate.

Table 21 A: Odour Factor Intensity Scores for Total Aroma Isolate (The Globe Technique) - Expt. 14

Odour Factor (Expt. 10 (ii))		Intensity Rating (4 assessors)
Meaty	F1	4,4,4,4
overcooked veg	F2	4,3,0,0
toasted/burnt	F3	3,4,3,2
ammonia-like	F4	0,0,2,0
oily/fatty	F5	2,0,0,1
spicy/fragrant	F6	0,0,0,3
musty/mouldy	F7	1,0,0,3
flat/dull	F8	2,3,0,0
buttery	F9	2,1,1,3

Table 21 B: Odour Quality Intensity Scores for Total Aroma Isolate (The Globe Technique) - Expt. 14

OQ	Intensity Rating (4 assessors)
meaty raw	0,0,0,0
meaty boiled	2,1,0,2
meaty roast	4,4,4,4
ammonia-like	0,0,2,0
animal/goaty	0,0,0,0
blood-like	1,0,0,0
broth-like	0,1,2,4
burnt	4,4,4,2
buttery	2,1,2,3
cooked cabbage	4,2,0,0
vegetables - overcooked	4,3,0,0
cool, cooling	0,0,0,0
earthy/soil	1,0,2,0
flat/dull	2,3,0,0
fragrant	0,0,1,3
herbal/hay	0,0,0,0
musty/mouldy	0,0,0,2
oily/fatty	2,0,1,1
paint-like	0,0,0,0
sharp/pungent	0,1,3,0
spicy	0,0,0,2
sweaty	0,0,0,0
sweet	0,0,0,4
toasted	3,3,4,4

The odour factor sensory analysis shows that the total isolate has the following main characteristics:-

The Meaty Factor (F1))
The Toasted/Burnt Factor (F3)) strong characteristics

The Cooked Vegetables Factor (F2))
The Buttery Factor (F9)) moderately strong characteristics
The Flat/Dull Factor (F8))

The Oily/Fatty Factor (F5))
The Fragrant Factor (F6))
The Musty/Mouldy Factor (F7)) weak characteristics
The Ammoniacal Factor (F4))

From the OQ analysis, specific sensory properties of the roast beef aroma isolate may be defined as:-

(i) The selected 2ℓ globe was partially evacuated by removing 500cm^3 of its contents using the apparatus depicted in Fig.29 p.89 . The sampled Tenax tube, obtained as in Expt. 2g, and connected as shown at position A, was flushed with dry N_2 at 30ml min^{-1} for 3.5 min,heat desorbed, under the conditions described in Expts. 1b and 2c, and then the volatiles flushed by N_2 into the globe for 13 min. after which time the globe was restored to atmospheric pressure. Four assessors sniffed the aroma at the odour ports, with the N_2 flow maintained, and described the perceived aroma in their own words.
(ii) The 2ℓ glass globe was silylated by the addition of 1ml Silyl-8 at 200°C with a dry N_2 flow of 30ml min^{-1} for 2h. Heating the globe during silylation and also during Tenax desorption and sensory analysis was achieved by winding 0.6m heating cord (Electrothermal Series HC) around the globe and attaching it to a Variac controller. A setting of 190v maintained the temperature within the globe at 200°C indefinitely and free of fluctuations. Insulation was achieved with glass wool enclosed by aluminium foil. The above experiment was repeated with the following variables changed in order to compensate for the effect of heating. Partial evacuation was increased to 1.2ℓ and the desorbed volatiles from the Tenax tube were flushed into the globe for 12 min at 30ml min^{-1}.

EXPERIMENT 14: Sensory Analysis of the Total Aroma Isolate (the Globe Technique).

(i) Using the apparatus shown in Fig.30,p.89, the 2ℓ. silylated glass globe, insulated by glass wool and enclosed in aluminium foil, was heated to 200°C by means of a Variac controller set at 190V. The odour ports and the tap at (C) were closed. The vacuum pump was attached to the globe via taps (j) and (f2) for 10s, then switched to atmosphere. Evacuation removed 1.2ℓ. as measured by the volume of water displaced.

The double oblique tap (f_1) was switched to atmosphere whilst setting the required N_2 flow of 30ml min^{-1}, measured by a flowmeter (g) and constantly monitored. This tap, and the on/off tap (c), were then opened for 5s to flush the air in the Tenax tube (e), sampled under conditions of Expt. 4b, into the globe by the N_2 flow. The double oblique tap (f1) was again switched to atmosphere and the heat desorption procedure commenced. Heating the Tenax at 250°C was maintained for 1 min. The tap at f_1 was then re-opened and the desorbed volatiles

	strongly	meaty, roast (F1, F3)
		burnt (F3)
		toasted (F3)
	moderately	buttery (F9)
		cooked cabbage (F2)
		vegetables, over-cooked (F2)
		broth-like (F1)
		meaty,boiled (F1,F2)
		flat, dull (F8)
and	weakly	fragrant (F6)
		oily, fatty (F5)
		sharp, pungent (F4)
		sweet (F2, F9)
		earthy, soil-like (F4,F5)
		ammonia-like (F4)
		blood-like (F1,F2,F5,F7)
		musty, mouldy (F7)
		spicy (F6)

The factor affiliations are in good agreement in both analyses. It is not possible to compare these results directly with those from the GC technique (Expt. 12) because of the heat desorption-caused partitioning effect. However, meaty-roast predominates in the 'total' cooked beef aroma isolate. In Expt. 12 it was shown that meaty-boiled (and associated OQ's) dominated the N_2 pre-flush period and meaty-roast (and associated OQ's) the flush-on period. This adds weight to the view that higher boiling components are responsible for characteristic roast notes[229].

The novel approach to the problems of sensory analysis of aroma isolates has been successful. The advantages of the Globe technique were firstly that it acted as a mixing chamber such that the partitioning effect noted in the GC technique was overcome. The'total' aroma sample also retained its particular aroma qualitatively in the globe over a relatively long period of time and therefore the technique is suitable for a panel of assessors. Transfer of volatiles from the Tenax tube into the globe achieved the aim of qualitative transferral.

EXPERIMENT 15: Gas Chromatographic Odour Port Assessment of Individual Aroma Components.

The combined results from the 4 assessors for the OPA of the roast beef (1h) aroma isolate are presented in Table 12, p. 71. Despite the problems inherent in OPA, it is a valuable technique for locating those parts of the chromatogram of sensory interest; areas of meaty character are cross-hatched in Fig. 31. As found by other workers[235] these 'peaks' are small, occur in valleys between peaks or as unresolved shoulders of large peaks. Further investigation of the 31 areas described in OPA as meaty reveals that only 9 GC/MS identifications were obtained for this particular isolate. These were 3-methylbutanal, hexan-1-al, butan-1-ol, pentan-1-ol, dimethylsulphide, 2,4,5-trimethyl-3 oxazoline, a C_4 amine, chlorobenzene and octamethylcyclotetrasiloxane.

3-Methylbutanal has been found to be an important contributor to roasted beef aroma[27,190]. In this study it represents 3.5% of the total peak area and was described as toasted bread, meaty and sweet. Hexan-1-al has been described as slightly meaty as well as green beans, cooked vegetables, oily and fishy. It represents <1% of the total peak area and has also been described as green[264] and green, sickly and pungent[47].

Alcohols are not generally recognised as possessing meaty qualities and the presence of butan-1-ol and pentan-1-ol in sensorially defined meaty areas may be co-incidence i.e. the compounds actually responsible for the meaty aromas co-elute with the alcohols but remain unidentified. These two alcohols represent <1.5% of the total peak area.

Dimethylsulphide represents a relatively large proportion of the total peak area (5.5%) and was described as faint warm rubber as well as meaty. It has been described as sickly and sulphurous and found to be an important contributor to the burnt quality of canned beef stew[47]. It also has synergistic properties[265] and is an effective modifier of flavour quality[161]. The 2,4,5-trimethyl-3-oxazoline, described here as burnt, roast cereal, meaty, roast meat, charred, dripping and pork represents just 0.05% of the total peak area. First identified by Chang et al. in boiled beef, it was originally thought to possess a characteristic boiled beef aroma[148]. The C_4 amine of MW73 was described as meaty roast and pleasant and represented 0.20% of the total peak

area. In general, amines possess unpleasant, fish-like, ammoniacal qualities[266].

Chlorobenzene, identified here for the first time in cooked beef aroma, represents 0.1% of the total peak area. Although described as meaty, roast, lamb, gravy, savoury, smoky, buttery and oily/fatty it is unlikely that it is actually responsible for meatiness. This is probably the same for octamethylcyclotetrasiloxane, i.e. unidentified co-eluting compounds are probably responsible for these meaty areas.

A remaining 22 meaty areas indicate that compounds responsible for cooked beef aroma are present but at levels below the detector /identification limits. It is possible that the 4h aroma sample may have afforded identifications in these areas. This possibility is explored in Part III.

The acetone/carbon disulphide, methanethiol and dimethyltrisulphide peaks represent 26%, 11% and 10% of the total peak area respectively. Thus each compound has considerable influence on the aroma of the cooked beef sample, although none of these aliphatic sulphur compounds possesses specifically meaty qualities. In this project acetone/ carbon disulphide was described as chemical, solvent-like and metallic, methanethiol as rotten vegetables, faecal and bad eggs, and dimethyltrisulphide as smoky, sweet, fruit and chemical solvent. The latter two have been identified in canned beef aroma and described as sickly, sulphurous, cooked cabbage and sulphurous, burnt, cooked cabbage respectively[47]. The combination of diacetyl and acetoin, which has been found to give a buttery note to canned beef stew[22] and boiled beef[23], was identified in this sample and described as buttery, toffee, caramel, butterscotch, sweet and baked cakes. Buttery qualities are also contributed by pentan-2-one and octan-1-ol.

Correlation of these OPA results with identities obtained from the 4h roast beef aroma isolate is considered in Part III.

EXPERIMENT 16: Fractionation of 'Total' Aroma Sample and Sensory Analysis of fractions using
a) the GC Technique and
b) the Globe Technique.

(a) The descriptive results obtained using the GC technique for the two fractions containing small and large peaks respectively, were examined as described in Expt. 12 and individual assessors' difference between descriptor scores compiled. A difference was said to occur if perceived by

3 assessors for any particular OQ. The results are presented in Table 22 .

The results show the large peak fraction (LPF) to possess more meaty-boiled and broth-like character than the small peak fraction (SPF) in the N_2 pre-flush period. Of the lower boiling compounds which are desorbed by the ambient N_2 pre-flush and therefore dominate this period, meaty areas are mostly associated with shoulders of large peaks (see Fig.31+32A, p.124)

During the flush-on period however, the SPF was found to possess more meaty-boiled and meaty-roast character than the LPF. Higher boiling components predominate in this period and from Fig.31+32B it can be seen that meaty areas mostly co-incide with the small peaks or those sections of the chromatogram designated as the SPF.

(b) The summarised results of the sensory analysis of the aroma fractions using the Globe Technique are shown in Table 23 . Individual assessors responses were compiled as described in (a). The results show higher scores in the LPF for meaty-boiled and meaty-roast OQ's. This may be due not only to the meaty areas associated with large peak shoulders but also the very close proximity of some SPF meaty areas to peaks designated as the LPF (See Fig. 31, p.124) The non-ideal fractionation would account for this.

Results (a) and (b) show that sensory differences between the two aroma fractions were detected. It had been hoped, however, that a cleaner division of meaty character might have been obtained.

Table 23 : Summarised Results of Sensory Analysis of SPF and LPF Using the Globe Technique - Expt. 16(b)

		Factors	Odour Qualities
SPF	described as	meaty* buttery* toasted/burnt oily/fatty*	meaty-boiled* meaty-roast broth-like buttery* fragrant* sweet* burnt o'cooked vegetables oily/fatty
LPF	described as more	meaty* spicy/fragrant*	meaty-boiled* meaty-roast* broth-like* fragrant* spicy*
LPF	described as less	buttery	buttery sweet*
LPF	described as equally	toasted/burnt oily/fatty	burnt toasted oily/fatty o'cooked vegetables

SPF = Small Peak Fraction
LPF = Large Peak Fraction

Table 22 : Summarised Results of Sensory Analyses of Small and Large Peak Fractions (SPF and LPF) Using the GC Technique - Expt. 16a

	N_2 Pre-Flush Period		Flush-on Period	
	Factors	OQ's	Factors	OQ's
SPF DESCRIBED AS:-	meaty o/cooked vegetables* NH_3-like/pungent oily/fatty* musty/mouldy* buttery*	meaty-roast meaty-boiled broth-like cooked cabbage* o/cooked vegetables* NH_3-like animal/goaty* oily/fatty* musty/mouldy* sweaty* buttery* sweet* faecal* urine*	meaty* toasted/burnt* spicy/fragrant* musty/mouldy* buttery	meaty-boiled* meaty-roast* broth-like* burnt* toasted* spicy* fragrant* musty/mouldy buttery sweet* green peppers* rubbery* charcoaled steak*
LPF DESCRIBED AS MORE:-	meaty	meaty-boiled* broth-like*	buttery*	buttery* sweet* smoky bonfires
LPF DESCRIBED AS LESS:-	buttery* o/cooked vegetables* musty/mouldy oily/fatty	buttery* cooked cabbage* o/cooked vegetables* musty/mouldy oily/fatty sweaty* urine faecal	meaty* toasted/burnt*	meaty-boiled meaty-roast* broth-like musty/mouldy spicy rubbery
LPF DESCRIBED AS EQUALLY:-	NH_3-like/pungent	NH_3-like animal/goaty sweet* meaty-roast	spicy/fragrant* musty/mouldy*	fragrant* green peppers* charcoaled steak burnt* toasted*

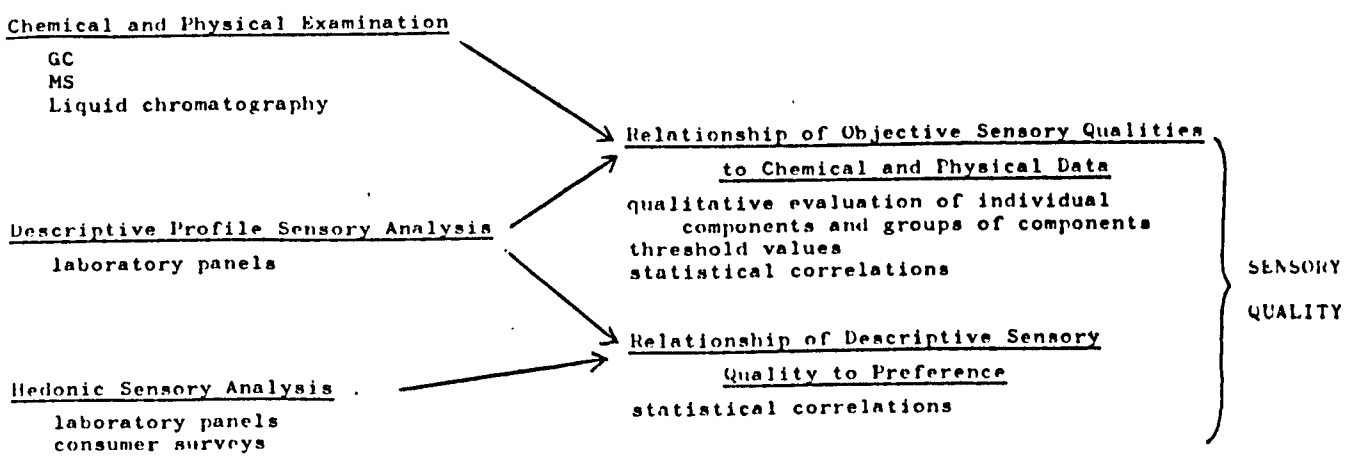
* - represents high intensity of an OQ

PART III: CORRELATION OF CHEMICAL AND SENSORY DATA

INTRODUCTION

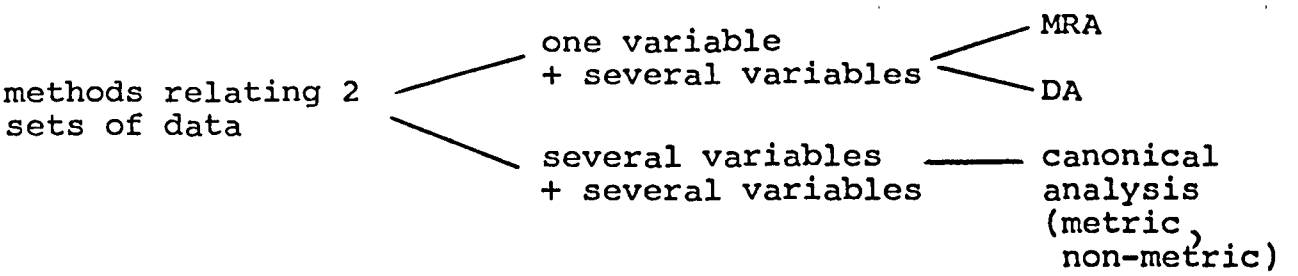
Parliment and Scarpellino stated that the ultimate goal in aroma research is "to establish the identity and importance of the volatile flavour components in a flavour essence"[242]. Achievement of this goal is difficult because the aroma response is integrated whereas that for GC is differential[267]. Since the chemical and sensory results are obtained from two different disciplines, the problems and errors involved in each are very different[268]. As pointed out by Noble[269], the chain is as strong as its weakest link e.g. the problems involved in obtaining aroma isolates with characteristic aromas, obtaining chromatograms representative of the original food volatiles and the inherent weaknesses in using humans as analytical instruments in sensory analysis have all been described. Thus if practical correlations are to be possible these errors must be minimised[270].

The methods used for the evaluation of sensory quality of a food (chemical/physical and sensory properties) have been summarised by Williams[271].



Drawing from several reviews of correlation methods for data of this type, approaches are, in general, purely statistical relying on multivariate techniques such as multiple regression analysis (MRA), discriminant analysis (DA), canonical and covariance analysis, to relate two sets of data [235,272-274]. Multivariate analysis describes statistical techniques involving

several variables, even if only one dependent variable is considered at a time. The following diagram demonstrates the statistical approaches[272].



The three major reasons for carrying out correlation of sensory data with chemical/physical measurements are (i) classification, (ii) prediction, and (iii) future formulation[235]. The correlations obtained can be categorised into (a) ad hoc relations - ungeneralisable relations with no predictive power, (b) predictive relations which hold true in all sets of certain type of data, and (c) causative relations which contain valid 'stimuli-response connections' and as a consequence have high predictive and explanatory power[275]. The statistical correlations attempted in aroma research, because of their varied aims, have been performed on many different combinations of sensory and chemical data, e.g. preference scores[276], flavour scores[277-279], odour descriptors[75,280,281] (for the sensory data) and total GC peak area[282,283], individual peak areas[243,246,284,285], peak ratios[286] (for the chemical data).

Before the advent of computers, correlations performed were relatively simple and often visual. Nearly 100 years ago, Galton developed the "index of co-relation" thereby introducing correlation of two sets of data[287]. Crucial to correlation today however are multivariate statistics enabled by high-speed computers[235]. Stepwise Discriminant Analysis (SDA) and Stepwise Regression Analysis (SRA) feature as the most widely-used methods for which there are several statistical packages available e.g.[262,288]. SDA serves to segregate from a set of variables those variables possessing the greater discriminating power, i.e. its main purpose is to classify and in aroma research it has been employed to achieve varietal classifications of wines[76], beers[289], sweet corn aromas[290], soy sauce[291], teas[292] and coffees[293].

On the other hand, MRA (including SRA) is a general statistical technique enabling the analysis of the relationship

between a dependent variable and a set of independent or explanatory variables. Regression can be used as a descriptive tool, such that the dependence of one variable on others is summarised and broken down, or as an inferential tool by means of which relationships in the population are evaluated from the examination of sample data. The most important uses of the technique as a descriptive tool are (i) to find the best linear predictor equation and evaluate its explanatory power and predictive ability, (ii) to control for other confounding factors in order to evaluate the contribution of a specific variable or set of variables, and (iii) to find structural relations to provide explanations for seemingly complex multivariate relationships.

The predictive ability of regression equations is the most desirable feature of MRA and SRA for aroma researchers. Some of the regression analyses used in aroma research have been relatively simple in that just one or two independent variables have been used to predict the sensory score[282,283]. Further presentation will be restricted to discussion of MRA and SRA (a form of MRA) and noteworthy applications of these techniques to food aromas.

MRA utilises a parametric approach to sensory data based on several assumptions which imply that the sensory attributes of interest are perceived as 'continua' with respect to magnitude of intensity, which in turn can be estimated in terms of numbers and that the distribution of the numerical response data obtained follows some parametric distribution function (notably normal or log normal)[294]. Careful data selection, both chemical and sensory, is vital if spurious correlations are to be avoided[235,295]. Also if the number of sensory variables is limited, the likelihood of colinearity between variables is reduced and thus regression efficiency is maximised[246]. With regard to the abundance of GC data, Powers has assessed the problem to be in the selection of relevant information from that available[296]. By using this data, the regression equation serves to sum up all the evidence of a large number of observations in a simple statement, expressing in a condensed form, the extent to which the differences in the dependent variable tend to be associated with differences in each of the other variables. Regression equations may be linear, quadratic, cubic or higher order functions, the first

being the simplest and most widely used. For MRA the linear model is:

$$Y = \beta + \beta_1 X_1 + \beta_2 X_2 + \ldots\ldots + \beta_M X_M + e_i$$

where Y = dependent variable
X_i = independent or explanatory variable
β = regression constant
β_i = regression coefficient
e_i = residuals (predicted Y' values fall along the regression line. The vertical distances $(Y - Y')$ of the points from the line represent the residuals or errors of prediction)

By its selection of the regression constant β, and regression coefficients β_i, the regression strategy minimises the sum of the squared residuals, $\sum_{i=1}^{N} e_i^2$, ie. $\sum_{i=1}^{N} (Y_i - Y_i')^2$. Thus, by using the least squares criteria the line of best fit or regression line is found. Multivariate techniques currently comprise 4 methods of finding the best regression line (a) all-possible regressions procedure, (b) backward elimination, (c) forward selection, and (d) stepwise regression procedure. Both (b) and (c) use partial F-statistics to test the significance of an explanatory variable to be included or deleted[252]. Statistically the criterion of a good regression equation is one in which the corrected coefficient of multiple determination, $\bar{R}^2$, is maximised (a coefficient of 1 represents complete agreement). The F-ratio also provides a measure of significance[297]. Significance, however, does not infer good interpretation or predictive ability. Before a variable can truely be said to be correlated to aroma, its effect on aroma caused by its addition or deletion must be established. This effect must occur in the predicted direction by the predicted extent. Causality can not necessarily be inferred from a regression analysis but a meaningful interpretation of the relationship between variables can be described in a statistical sense[252]. Despite the pitfalls and the need for caution, valuable insights have been provided concerning interrelations and correlations using regression techniques.

Of particular note is the work by Aishima and Nobuhara on soy sauce aroma[285]. GC peak areas were selected as explanatory variables and after transforming them to arc-sine

and log functions (previously proven useful) they were regressed onto preference scores obtained from 150 assessors. Using an all-possible subsets regression, the resulting linear relationship showed a highly significant correlation. It was shown that the quality of soy sauce could be evaluated objectively from the GC data. Also, the selection of peaks as explanatory variables was important for the accuracy of estimation, as accuracy increases with increased input of variables in the regression model. Aishima performed a rigorous FA (principal components analysis) on the 39 selected GC peaks and regressed the resulting 5 factors onto the sensory scores[284]. $\bar{R}^2$ was 0.788 and a highly significant F-value was obtained.

Using GC data and sensory descriptors obtained for wine, Noble performed a 2/way analysis of variance to eliminate GC peaks not contributing significantly to the differences between wines[75]. SRA was then applied to the remaining GC peaks for each OQ. Thus 80 GC peaks from headspace analysis were reduced to 20 peaks for SRA. While significant equations were obtained within the data set, results were said to have no predictive power unless similar wine samples were used.

GC data (13 explanatory variables), from a Likens-Nickerson extraction of canned peaches, and intensity scores for 8 descriptors finalised by assessors were analysed by SRA[280]. For the intensity scores of 'overall peach odour', 3 peaks were found to account for 95.4% of the variation. All other regression equations obtained, excepting one, had high R^2 (not corrected for degrees of freedom). Williams and Applewhite used the flavour scores and GC data of the volatile oil of soybean for SRA and obtained $\bar{R}^2$ from 0.65-0.77[277]. This confirmed the above conclusion of Aishima that $\bar{R}^2$ increases as the number of explanatory variables in the equation increases, but they added that if the number of samples was small then increased $\bar{R}^2$ may be spurious and the increase must be tested for significant difference from zero.

The only citations referring to correlation of "objective-subjective" data obtained for cooked beef aroma are those by Persson and von Sydow[47,275]. They used GC and sensory data obtained from canned beef stew and applied various psycho-physical relationships. Mathematical models based on linear summations of the GC peak heights were formulated. The

validity of models was statistically tested by linear regression analysis and the goodness of fit of the regression models measured by the Chi-square statistic. Compounds found to be of importance to the burnt OQ are, in decreasing order:- methylpropanal, dimethylsulphide, methanethiol, 2-methylbutanal, 3-methylbutanal and H_2S. Many potential causative relations were found but were not claimed as such for reasons including the increased likelihood of these relations if the aroma is separated into OQ's. In addition, Persson et al. found a positive correlation between preference value and meaty (cooked) and fragrant OQ's and a negative correlation for preference value with sickly, musty/mouldy, animal/goaty and nasty.

Choice of Methods Used in This Project.

SRA was selected for correlating the chemical and sensory data obtained previously in these laboratories[1,3,5]. As well as being the most appropriate of the multivariate techniques, both types of data were suited to this analysis.

Non-statistical comparisons of the results of PartI and II of this thesis are also made in this chapter.

ATTEMPTED METHODS AND RESULTS AND DISCUSSION

(a) Statistical Correlation of Previously Published Data[1,3,5]

The 66 identified compounds were grouped into 11 chemical classes (see Table 24, p. 112) and the relative percentage abundance (RPA) of the individual peaks were summed within each class for each of the 14 differently-cooked beef aroma isolates. These 11 summed RPA values, representing the relative concentrations of e.g. alkanes, furans etc. in each isolate, were the explanatory or independent variables (X) for the regression. Grouping into chemical classes was, in effect, a data reduction procedure, since 66 explanatory variables were clearly inappropriate.

The dependent variables (Y) were the factor score averages

Table 24: Classification of Compounds Identified in Beef Aroma Isolates[1,3,5]

Alkanes
n-heptane
2-methylheptane
n-octane
n-nonane
n-decane
n-undecane
n-tetradecane
n-pentadecane
n-hexadecane
n-heptadecane
tetrachloroethane
a long chain hydrocarbon

Benzenoid Compounds
benzene
toluene
p-xylene
o-xylene
n-propylbenzene
a C_3 benzene
styrene
benzaldehyde
phenol
m-tolunitrile
ethylbenzaldehyde

Pyrroles
pyrrole
a methylpyrrole(?2-)
a dimethylpyrrole (2,5- or 2,4-)

Pyridines
a methylpyridine(??-)
a C_3 pyridine(?2-n-Pr)

Alkanes
dimethylbutene
hex-1-ene
hept-1-ene
oct-1-ene
non-1-ene
dec-1-ene
undec-1-ene
undec-4-ene
dodec-1-ene
tridec-1-ene
tetradec-1-ene
hexadec-1-ene
heptadec-1-ene
a C_{20} branched alkene (MW280)
a C_{20} branched alkene (MW280)
a C_{20} branched alkadiene (MW278)

Aldehydes
3-methylbutanal
hexan-1-al

Ketones
acetone
butanone
pentan-2-one

Furans
2-methylfuran
2-ethylfuran
2-n-pentylfuran

Sulphur Compounds
ethylmethylsulphide (?)
dimethyldisulphide
2-methylthiophen (?)

Alcohols
a methylbutanol
n-nonanol

Pyrazines
methylpyrazine
a dimethylpyrazine (prob. 2,6-)
ethylpyrazine
an ethylmethylpyrazine (2,6-?)
an ethylmethylpyrazine (2,5-?)
a trimethylpyrazine (2,3,5?)
a dimethylethylpyrazine (2,3,5?)
a dimethylethylpyrazine (2,5,3?)
a C_5 satd, substd. pyrazine (Me iso Bu-?)

(of the 15 trained assessors) for each of the 9 factors extracted by the FA described in Expt. 10(ii) for each of the 14 beef aroma isolates. A factor score, calculated for a particular sampling unit, (in this study a beef aroma isolate) is a specific value of a factor formed as a weighted sum of its explanatory variables (chemical classes in this case) i.e. it indicates how the factor depends on its explanatory variables[252]. Therefore a factor score expresses the degree to which each aroma isolate possesses the overall sensory properties defined by that factor. Interrelation between individual OQ's had already been accounted for by the FA and therefore the use of factor scores in SRA (where interrelation between dependent variable is undesirable) is an advantage.

A SRA was performed on the above data by the University of California BMDP9R Computer Program using the All-Possible-Subsets Routine[262]. The task is to explain the sensory variance in terms of the chemical data. Thus the contribution of the explanatory variables to this variance is the focal point of the analysis. Using the least-squares method for finding the regression line (p.109) guarantees that the sum of squares of residuals (unexplained variance) is minimised and independent of the predicted Y value, Y'.

i.e.

$$\sum (Y-\bar{Y})^2 \quad = \quad \sum (Y'-\bar{Y})^2 \quad + \quad \sum (Y-Y')^2$$

total variance explained variance unexplained variance

The coefficient of multiple determination, R^2 is an important measure of the proportion of the variation of the dependent variable determined by the variation of the explanatory variables. As $R^2 \longrightarrow 1$, the better the performance of the explanatory variables. R^2 is also a measure of the accuracy of the predictor equation or the strength of the linear association. When this value is corrected for degrees of freedom, R^2 becomes the corrected coefficient of multiple determination, $\bar{R}^2$. A more realistic indication of the linear association between explanatory variables and the dependent variables is obtained from $\bar{R}^2$ than R^2 as the latter effectively inflates the goodness of fit of the regression equation.

The All-Possible Subsets Routine, 9R, estimates regression equations for all combinations of explanatory variables. The

best equations are selected by the following criteria; lowest Mallows Cp or highest $\bar{R}^2$, the latter used in this analysis. Regression equations are of the form:

$$Y = \beta + \beta_1 X_1 + \beta_2 X_2 + \ldots \beta_m X_m + e_i$$

where Y = dependent variable
X_i = explanatory variable
e_i = residuals (unexplained variance)

β_i, the regression coefficient, is the slope of the regression line and indicates the expected change in Y' with a change in one unit of Y_i. The regression constant, β is the y-intercept of the regression line and represents Y' when $X_i = 0$.

The reliability of the regression coefficients is influenced by (i) the number of cases, (ii) standard error of the estimate, and (iii) the degree of independence of X_i, such that the more interrelated the explanatory variables, the less reliable the regression of Y upon any explanatory variable. Confidence limits are set up for the regression coefficients. When a sample size is relatively small, regression coefficient estimations follow the t-distribution. The t-value or T-statistic is calculated and reference to statistical tables gives the cut-off point for significance of the regression coefficients for the particular sample size. For 14 samples, the T-statistic is $\pm$ 1.96 for significance at the 5% level.

Table 25 summarises the results for the solutions obtained from the SRA of the 9 factors.

Of the original factors explaining > 5% total sensory variance (F1 — F6), only 2 have $\bar{R}^2 > 0.5$ and 2 have no solution at all. The 3 factors explaining < 5% total variance each have $\bar{R}^2 > 0.5$. According to Williams and Applewhite the following ranges represent the goodness of fit of a regression line[277]:

excellent fit if $\bar{R}^2$ is 0.76 - 1.00
good fit if $\bar{R}^2$ is 0.51 - 0.75
fair fit if $\bar{R}^2$ is 0.26 - 0.50
no correlation if $\bar{R}^2$ is 0.01 - 0.25

Factor 1 - The Meaty Factor

From the above levels, $\bar{R}^2 = 0.812$ represents an excellent fit for the regression equation obtained.

Table 25 : SRA Results for 9 Factors.

FACTOR	Explanatory Variable	Regression Coefficient and Constant	T-Statistic	R^2
1. The Meaty Factor (explains 15.8% total variance)	alkenes	0.011	3.42	0.812
	alcohols	0.038	2.82	
	ketones	0.027	3.46	
	furans	0.029	4.29	
	pyrazines	0.025	5.22	
	intercept	-0.754		
2. The Cooked Vegetables Factor(explains 10.7% total variance)		NO SOLUTION		
3. The Toasted/Burnt Factor (explains 8.7% total variance)	aldehydes	0.003	3.00	0.608
	ketones	0.018	2.47	
	furans	0.022	4.43	
	pyrroles	-0.073	-2.32	
	pyridines	-0.025	-2.99	
	intercept	-0.135		
4. The Ammoniacal Factor(explains 7.8% total variance)	alkenes	-0.007	-1.92	0.349
	benzenoids	0.012	2.86	
	intercept	-0.088		
5. The Oily/Fatty Factor(explains 6.9% total variance)		NO SOLUTION		
6. The Fragrant Factor(explains 5.5% total variance)	alkanes	0.018	2.00	0.385
	alcohols	-0.048	-1.99	
	furans	-0.027	-2.19	
	pyrazines	-0.032	-2.97	
	intercept	0.358		
7. The Musty/ Mouldy Factor (explains 4.9% total variance)	aldehydes	0.004	4.98	0.786
	ketones	-0.008	-2.32	
	sulphur compounds	0.018	3.98	
	benzenoids	0.007	3.55	
	furans	-0.014	-3.24	
	pyrazines	0.006	2.22	
	intercept	-0.193		
8. The Flat/Dull Factor(explains 4.7% total variance)	ketones	0.022	3.64	0.547
	pyrroles	-0.120	-3.75	
	pyridines	-0.033	-3.83	
	intercept	0.051		
9. The Buttery Factor (explains 4.4% total variance)	alkanes	-0.042	-5.82	0.881
	alkenes	-0.043	-6.53	
	alcohols	-0.029	-1.69	
	aldehydes	-0.029	-6.31	
	sulphur compounds	-0.181	-3.88	
	benzenoids	-0.047	-8.39	
	furans	0.031	5.04	
	pyridines	-0.033	-3.26	
	pyrazines	-0.031	-3.99	
	intercept	1·743		

This factor defines general meaty qualities and does not discriminate between raw, boiled and roasted aromas. Other associations are broth-like, blood-like, animal-goaty, sweet and earthy qualities. These are therefore explained by the combined effects of the alkenes, alcohols, ketones, furans and pyrazines on p. 115. Although OPA does not take account of odour interactions in complex mixtures, it is likely that the following representatives (or their co-eluents) are significant contributors to this factor, as evidenced from their OPA descriptions[248].

dimethylbutene (sweet)
hex-1-ene (meaty)
tridec-1-ene (earthy)
hexadec-1-ene (animal-like)
a C_{20} branched alkene (MW 280) (meaty)
butanone (meaty)
2-ethylfuran (sweet)
2-n-pentylfuran (meaty and sweet)
methylpyrazine (sweet)
ethylpyrazine (sweet)
a dimethylpyrazine (2,6-?) (meaty)
an ethylmethylpyrazine (2,5-?) (sweet)
a dimethylethylpyrazine (2,5,3-?) (meaty)
a trimethylpyrazine (2,3,5-?) (earthy)
a C_5 sat^d. sub^d. pyrazine (meaty)

Factor 1 can be expressed by:

Meaty Factor = -0.754 + 0.011 alkenes + 0.038 alcohols
+ 0.027 ketones + 0.029 furans
+ 0.025 pyrazines.

Factor 2 - The Cooked Vegetable Factor

No solutions were obtained with significant T-statistics for this factor. This is surprising as F2 explains 10.7% of the variance in the cooked beef aroma samples. Also the OQ's highly associated with this factor do not load onto other factors. However, less closely-associated OQ's such as blood-like, sweet and meaty boiled have a complexity > 1. Thus despite the orthogonal rotation in the FA some interrelation between factors remains.

Factor 3 - The Toasted/Burnt Factor

The associated OQ's i.e. toasted, burnt, meaty-roast, are highly correlated with this factor.

These are explained mainly by the combined effects of the aldehydes, ketones and furans on p. 115. Specific compounds

within these classes which possessed burnt/roasted OPA descriptions are 3-methylbutanal and 2-ethylfuran. The negative coefficient for the pyrroles was, at first sight, surprising since burnt/smoky OPA descriptions had been obtained for every pyrrole identified. However the literature states that whilst many pyrroles give burnt aromas in concentration, their odours on dilution are very different - often nutty, corn-like and bread-like[298]. Also, more importantly, synosmic effects do occur i.e. two or more compounds together give a combined sensation which is very different from that of any single component[10]. After all this was the purpose of the present exercise.

$\bar{R}^2 = 0.668$ indicates a good fit for the regression line obtained. F3 can be expressed by:

Toasted/Burnt Factor = -0.135 + 0.003 aldehydes + 0.018 ketones
+ 0.022 furans - 0.073 pyrroles
- 0.025 pyridines.

Factor 4 - The Ammoniacal Factor

A fair fit was obtained for this regression equation, $\bar{R}^2 = 0.349$. This factor, chiefly characterised by the terms ammoniacal, animal/goaty and sharp/pungent is largely explained by the benzenoids of p. 112 . Based on OPA, styrene (solvent-like), phenol (solvent, medicinal) and m-tolunitrile (acrid) could well be significant here. Min et al. have also reported that many alkylbenzenes identified in roast beef have OPA qualities described as "like mothballs"[54]. The negative coefficient for the alkenes is not surprising since many have flat, dull, sweet and stale qualities[248]. The solution obtained expresses this factor by:

Ammoniacal Factor = -0.088 - 0.007 alkenes + 0.012 benzenoids

Factor 5 - Oily/Fatty Factor

Again a factor explaining > 5% total variance but for which no significant solution was obtained. The mixed nature of this factor (some OQ's have a complexity > 1) may have precluded a good solution.

Factor 6 - Fragrant Factor

The best regression line obtained for F6 had $\bar{R}^2 = 0.385$ i.e. a fair fit. F6 is closely associated with spicy, fragrant,

sweaty and herbal qualities. Alkanes are the main contributors and a sweaty OPA was obtained for tetrachloroethane[248]. Alcohols, furans and pyrazines detract from this factor. F6 can be expressed by:

Fragrant Factor = 0.358 + 0.018 alkanes - 0.048 alcohols
- 0.027 furans - 0.032 pyrazines

Factor 7 - Musty/Mouldy Factor

Seemingly an excellent fit ($\bar{R}^2$ = 0.786) was obtained for F7. Interpretation and its predictive power must be regarded with caution as this factor explains < 5% total sensory variance. This may be only random experimental variation. Some OPA descriptors do agree and these compounds may be significant: Hexanal (stale), o and p-xylenes (sickly, stale), a trimethylpyrazine (?2,3,5-) (earthy, stale). F7 can be expressed by:

Musty/Mouldy Factor = -0.193 + 0.004 aldehydes - 0.008 ketones
+ 0.018 sulphur compounds
+ 0.007 benzenoids - 0.014 furons
+ 0.006 pyrazines

Factor 8 - Flat/Dull Factor

This factor explained < 5% total variance in the FA, thus its predictive power is low despite $\bar{R}^2$ = 0.547. F8 can be expressed by:

Flat/Dull Factor = 0.051 + 0.022 ketones - 0.120 pyrroles
- 0.033 pyridines

Factor 9 - Buttery Factor

F9 explains < 5% total variance and therefore the regression equation obtained ($\bar{R}^2$ = 0.881) has low predictive power. F9 can be expressed by:

Buttery Factor = 2.743 - 0.042 alkanes - 0.043 alkenes
- 0.029 alcohols - 0.029 aldehydes
- 0.181 sulphur compounds - 0.047 benzenoids + 0.031 furans - 0.033 pyridines
- 0.031 pyrazines.

The furans on p. 112 are therefore important and OPA descriptions which agree with this were obtained for 2-ethylfuran (buttery, sweet, caramel, malty)[248].

Some of the above solutions had low $\bar{R}^2$ values. The poor explanatory power of these regression equations may be due to deficiencies in the original FA where some of the OQ's loaded onto more than one factor. This occured infrequently however. Interrelationship between variables serves to reduce the efficiency of regression[246]. Also the chemical data were obtained from aroma isolates whilst the sensory data derived from cooked beef samples. Thus, possible qualitative and quantitative changes, plus artefact formation occurring during the aroma isolation and concentration stages increase the possibility of the aroma isolate differing sensorially from the samples assessed during sensory analysis, despite the fact that the isolates had aromas representative of cooked beef. Additionally, unidentified compounds and any co-eluting compounds would confound the regression.

Nevertheless two efficient regressions were obtained for F1 (Meaty Factor) and F3 (Toasted/Burnt Factor). Reference to Table 26, which contains the standardised coefficients for these two regressions, shows the effect or weight each explanatory variable has in the regression equation. Effectively the standardised coefficient gives an indication of the sensitivity of Y to X_i i.e. for a shift of one standard deviation in a particular X_i, there will be a change in Y equal to the amount of the standardised coefficient for that particular X_i. It can be seen for the Meaty Factor that the pyrazines and furans have greatest influence in the regression equation. For the Toasted/Burnt Factor the furans, ketones and aldehydes have the largest effect on the equation. Other classes which may have been expected to appear in this regression equation because of their individual burnt/roasted OPA qualities are the sulphur compounds and the pyrazines.

Table 26: Standardised Coefficients for Regression on Factors 1 and 3.

FACTOR 1 - Meaty Factor		FACTOR 3 - Toasted/Burnt Factor	
Explanatory Variable	Standardised Coefficient	Explanatory Variable	Standardised Coefficient
pyrazines	0.909	furans	0.874
furans	0.642	ketones	0.732
alcohols	0.626	aldehydes	0.617
ketones	0.587	pyrroles	-0.636
alkenes	0.557	pyridines	-0.657

Chang and Peterson have included furanoid compounds and heterocycles as important contributors to cooked beef aroma[15], which supports the inclusion of the furans and pyrazines in the regression equation for the Meaty Factor, F1'. The alcohols, aldehydes and ketones were not classed by these workers as primary contributors but were included in the regression equation. Other workers however stress the importance of the aldehydes[4,27,47,192,300].

The only work of comparable nature concerned the aroma of retorted beef stew[47,275]. The application of regression models to chemical and sensory data for 35 samples suggested several causative relationships of 2-methylpropanal, methylbutanal, H_2S, methanethiol and dimethylsulphide for retort flavour. The SRA performed here however involved classes of compounds, and the regression equation for the Toasted/Burnt Factor included a positive contribution by the aldehydes.

(b) Results of Part I vs Results of Part II

Non-statistical means were also used to gather evidence of the sensory properties associated with certain peak combinations, to pinpoint them on chromatograms and hopefully to define the most important chemical contributors to desirable cooked beef aroma. Simplification of the complex aroma to more manageable levels was achieved by the fractionation technique described in Expt. 16 and complementary sensory analysis of the fractions was performed using the techniques described in Expts. 12 and 14. Three approaches were made to achieve this aim.

(i) Sensory differences between the N_2 pre-flush/heating and flush-on periods of heat desorption are shown in Tables 20A,B,C(91,B) Using the technique described in Expt. 16 the appropriate fractions of an aroma isolate, obtained as in Expt. 4b, were collected at the exit of a 0.3m empty silylated glass column. Gas chromatograms of the re-chromatographed fractions showed that the chemical compounds (and their co-eluants) which correlated with these sensory differences were as summarised in Table 27.

In general, peaks eluting after water (peak 28) were very considerably decreased in intensities (and many were absent) in the sample collected during N_2 pre-flush and heating stages of desorption. There were four exceptions, where the peak areas were not significantly diminished, ie. diacetyl, but-2-en-1-al, a methylpentanolactone and acetoin.

It is concluded that peaks eluting before 3-hydroxybutanone (peak 47) are present in greater concentrations in the collected N_2 pre-flush/heating period fraction and many are associated with several sweet, sickly, fragrant, meaty-boiled, meaty, buttery (very significant) and caramel aroma descriptions which characterised this 'fraction' in Expt. 12. From Expt. 15, the most important contributors to these particular sensory properties are likely to be 3-methylpentane, methylpropane (?) a C_4 amine MW73, 3-methylbutanal, butan-2,3-dione, but-2-en-1-al, a methylpentanolactone, 3-hydroxybutanone and 6 unidentified/unresolved components.

Peaks eluting after peak 47 are present at greater concentrations in the collected flush-on period fraction and many are associated with several earthy, burnt, charred, unpleasant, roasted, buttery (slight) smoky and meaty aroma descriptions which characterised this 'fraction' in Expt. 12.

From Expt. 15, important contributors to these sensory qualities are likely to be dimethyltrisulphide, octan-1-ol (?) and 13 unidentified/unresolved components.

Peaks eluting after peak 57 were not in sufficient concentration for detection, but possess several meaty, burnt, roasted, charred aroma descriptions assumed to contribute to the sensory properties peculiar to this fraction. In particular it is interesting to note that peak 14, described as buttery, sweet and popcorn is absent in the flush-on period fraction. The relatively large number of unidentifiable compounds in this fraction was unfortunate. However the evidence confirms the association of meaty, and in particular meaty-roast, qualities with relatively high boiling compounds present in trace quantities.

(ii) The sensory and chromatographic differences between the SPF and LPF (obtained in Expt. 16) are shown in Table 22 (p.105) and Figs.32A,B(p.124) respectively.

Compounds in the LPF which may account for the higher meaty scores during the pre-flush are trimethylethoxysilane, methanethiol, methylpropane, acetaldehyde, acetone, carbon disulphide, dimethylsulphide, a C_4 amine, propanal, methyl-propanal, methanol, ethanol, 1,1,1-trichloroethane, 3-methyl-butanal, pentan-2-one and dimethyltrisulphide.

During the flush-on period, the SPF was scored more highly for meaty qualities and the chemical compounds associated with this sensory difference are heptane, butan-1-ol, 2,4,5-trimethyl-3-oxazoline, a methylpentanolactone, hexamethyl-cyclotrisiloxane, pentan-1-ol, chlorobenzene, a C_7 hydrocarbon, octamethylcyclotetrasiloxane, 3-hydroxybutanone (acetoin), hept-3-ene, octan-1-ol and benzaldehyde. In fact peaks 37-56 and 58-65 (see Fig.32B, p.124) are exclusively in the SPF. The following may account for the more buttery sweet and empyreumatic notes associated with the LPF during the flush-on period - diacetyl, pentan-2-one and dimethyltrisulphide.

(iii) The OPA's of the 1h aroma isolate are discussed on pp. 102-103. As several compounds were common to both 1h and 4h chromatograms the meaty and roasted/toasted areas of the 1h aroma isolate chromatogram (see Fig. 31, p.124) could be correlated with the 4h aroma isolate chromatogram (see Fig. 33, p.124). The assumption made is that the concentration of

several compounds responsible for roast beef aroma, present but not identified in the 1h sample was sufficiently high in the 4h sample to afford identification. Thus when those areas of sensory interest detected in the 1h sample were examined in the 4h aroma sample chromatograms, information could be elicited as to the contribution of particular chemical components to desirable beef aroma.

Identifications of compounds correlated with meaty and roasted/toasted qualities are summarised in Table 28 .

Of these the following have been reported to possess meat-like qualities -

3-methylbutanal[248]
furan[299]
2,4-dimethyl-3-oxazoline[137]
2-furaldehyde[51]
2,4-dimethyl-5-ethylthiazole[137]

Similarly those compounds previously reported to possess specifically roasted/toasted character are -

a methylthiophen[10]
2,4-dimethyl-3-oxazoline[137]
2,4,5-trimethyl-3-oxazoline[137]
2-furaldehyde[51]
2,4-dimethyl-5-ethylthiazole[137]

These, combined with the above list are therefore likely to make significant contributions to roast beef aroma. Of the above only 3-methylbutanal and methylthiophen have previously been identified in conventionally-roasted beef aroma.

3-Methylbutanal is an important contributor to cooked meat aroma as are the aldehydes in general[4,27,47,192,300]. Both propanal and methylpropanal are of importance to the burnt odour of canned beef aroma[275]. Chang and Peterson however listed the aldehydes amongst classes which were not primary contributors to meat aroma on the basis that OPA of individual aldehydes yielded no meaty descriptions[15,139].

Furan and 2-furaldehyde are reported to possess caramel-like[298] and cooked, brown qualities[301] respectively. This agrees with the present results where they are present in GC areas described as both meaty and roasted. Ethylfuran and methylfuroate were also associated with roasted GC areas in this study. 2-Furaldehyde is believed to add a slight caramel-like character to the aroma of cooked beef[51]. Hodge et al.

Table 28 : Occurrence of Compounds in Meaty and Roasted/Toasted Areas of Beef Aroma Isolate Chromatograms.

Occurrence of Compounds	
Meaty Areas	Roasted/Toasted Areas
butan-1-ol[a]	
pentan-1-ol[a]	
propanal[b]	
methylpropanal[b]	
3-methylbutanal[a,b]	3-methylbutanal[a]
hexan-1-al[a]	
decan-2-one[b]	decan-2-one[b]
a C_4 amine[a]	a C_4 amine[a]
trimethylamine[b]	
a benzenoid[b]	
chlorobenzene[a,b]	chlorobenzene[a]
hexamethylcyclotrisiloxane[b]	
octamethylcyclotetrasiloxane[a]	
dimethylsulphide[a,b]	
ethanethiol[b]	
ethylmethyldisulphide[b]	
dimethyltrisulphide[b]	dimethyltrisulphide[a,b]
a thiophen[b]	
furan[b]	furan[b]
2- or 3-methylfuran[b]	
2-n-pentylfuran[b]	
2-furaldehyde	2-furaldehyde[b]
2,6-dimethyl-3-ethylpyridine[b]	2,6-dimethyl-3-ethylpyridine[b]
a dimethylethylpyrazine[b]	a dimethylethylpyrazine[b]
a C_5 substit. pyrazine[b]	
a thiazole[b]	
2,4-dimethyl-5-ethylthiazole[b]	2,4-dimethyl-5-ethylthiazole[b]
2-isopropyl-4-methyl-5-ethyl-thiazole[b]	
2,4-dimethyl-3-oxazoline[b]	2,4-dimethyl-3-oxazoline[b]
2,4,5-trimethyl-3-oxazoline[a,b]	
	diacetyl[a]
PLUS:-	pentan-2-one[a]
21 unidentified/unresolved components[a]	trimethylsilanol[a,b]
7 unidentified/unresolved components[b]	sulphur dioxide[b]
	a sulphur-containing compound[b]
	dimethyldisulphide[a,b]
	a methylthiophen[b]
	ethylfuran[b]
	methylfuroate[b]
	ethylmethylpyrazine[b]
	2,4,5-trimethyl-3-oxazoline[a,b]
	PLUS:-
	20 unidentified/unresolved components[a]
	13 unidentified/unresolved components[b]

Footnote: [a] represents occurrence in 1h aroma isolate

[b] represents occurrence in 4h aroma isolate

have classified the oxygen-containing heterocycles as possessing caramel, burnt sugar qualities[197]. In general, individual furanoid compounds which do not contain sulphur atoms possess aromas which are not meaty[228], although 2-methylfuran (identified in a GC meaty area) has been described as meaty and nutty[248]. Furanoid compounds are not considered important aroma components in cooked meats by Shibamoto[229] although the reverse is true for Chang and Peterson[15,139]. In combination with the benzenoid compounds they may contribute to the desirable qualities of well-cooked boiled beef[4]. Additionally, many furanoid compounds with sulphur substituents are known to possess meaty aromas[16] although none of these has yet been identified in natural beef aromas[199].

2,4-Dimethyl-5-ethylthiazole possess nutty, roasted and meaty qualities and, especially in view of its very low threshold value of 2ppb[137], is very likely to be an important contributor to roast beef aroma. The 2,4-dimethyl-3-oxazoline has been described as possessing nutty, vegetable aromas[137]. Several thiazoles and related thiazolines (i.e. sulphur/nitrogen heterocycles) have been described as meaty and/or roasted, while several oxygen/nitrogen heterocycles, i.e.oxazoles and oxazolines, have been described as sweet and green[137]. It has been claimed that thiazoles are more likely to contribute to the roasted, meaty character of beef than the oxazolines[137]. Increased substitution in the thiazoles confers added nutty, roasted and meaty notes[302] and it is interesting to note that several of the thiazoles identified in this study (some for the first time in beef aroma) were relatively highly-substituted members. Five other multi-heteroatomic compounds were identified in the 4h aroma isolate indicating their importance to well-cooked roast beef aroma.

The aliphatic sulphur-containing compounds, identified in both the meaty and roasted areas may contribute to the overall aroma of roast beef by virtue of synosmic effects; as they have low odour thresholds and their contribution to roasted qualities has been reported[3]. Dimethylsulphide contributes to cooked beef aroma[275], and dimethyltrisulphide in concentration as low as 10ppb significantly contributes to roasted chicken leg meat[303].

The contribution made by the 2,6-dimethyl-3-ethylpyridine is unknown as this is its first identification in a food aroma. However some pyridines have been associated with burnt and heavy

fruit aromas[4].

The two pyrazines identified in the meaty areas of the 4h aroma sample did not possess meaty qualities. This is the case for most pyrazines[16], their main contribution being to the aroma of roasted foods[229]. The identification of 34 pyrazines and 9 cycloalkylpyrazines in the basic fraction of roasted coffee supports their role in roasted food aromas, although not one was individually considered significant[304]. Thiophens are reported to be responsible for the mild sulphurous odour of cooked meat[305] and its roasted character[10]. Sulphides and thiophens were identified in the strong roast aroma produced from a heated model meat system[300]. Also, with certain carbonyls, sulphur compounds were implicated in the retort flavour[47] and burnt aroma[154] of canned beef. The importance of sulphur compounds to overall aroma of foods has been stressed by Schwimmer[207].

Schutte has suggested that heterocyclic compounds, sulphur and nitrogen-containing compounds and some phenols were important in meat aroma[10]. This study indicates the particular importance to meaty aroma of members of the aldehydes, especially 3-methyl-butanal, the furanoids, oxazolines and thiazoles. The odours possessed by the heterocycles, sulphur compounds, nitrogen-containing compounds and carbonyls indicate their significant contribution to the aroma of roast beef. Their presence in the 4h sample in comparatively large numbers (over half of the total number of compounds identified) clearly demonstrates their importance. In particular, 2,4-dimethyl-5-ethylthiazole, which possesses both meaty and roasted aromas and which was identified in both meaty and roasted/toasted GC areas, is very likely to be an important contributor to the aroma of roast beef.

CONCLUSIONS

Prior to this work, beef aroma research had generated much complex chemical data but produced little systematic correlation of such data with detailed sensory data which is necessary to establish the contribution of the chemical components to cooked beef aroma. In this project a new and improved approach to beef aroma isolation was developed. This involved a modified headspace technique in conjunction with adsorption (and simultaneous concentration) of volatiles onto Tenax GC, and the method gave cooked beef aroma isolates with good representative aromas. Tenax has not previously been used in meat aroma research and direct heat desorption of volatiles from the Tenax onto the GC column also represents a new approach in headspace analysis of beef aroma. Analysis of the aroma isolates by GC/MS, using EI and CI techniques, afforded a total of 78 identifications (see Tables 12 and 13, p. 71 and 72), listed for convenience in chemical classes below. Twelve compounds (in brackets) were identified for the first time in cooked beef aroma and those additionally marked * represent their first identification in any food aroma.

7 aliphatic hydrocarbons (hept-3-ene*)
6 aliphatic alcohols
7 aliphatic aldehydes
5 aliphatic ketones
3 carboxylic acids
1 lactone (a methylpentanolactone)
2 amines (trimethylamine, a C_4 amine)
3 benzenoid compounds
3 chlorine-containing compounds (chlorobenzene, tetrachloromethane*, 1,1,1 -trichloroethane)
5 silicon compounds
9 aliphatic sulphur compounds
2 thiophens
9 furanoid compounds (methylfuroate)
2 pyridines (2,6-dimethyl-3-ethylpyridine*)
5 pyrazines (3-isopentyl-2,5-dimethylpyrazine*)
6 thiazoles (2-isopropyl-4-methyl-5-ethythiazole, 2-propyl-4,5-dimethylthiazole*)
2 oxazolines
1 miscellany

Having sensorially validated the aroma isolation and GC analysis techniques, further detailed sensory analysis of aroma isolates and fractions thereof was accomplished using a simplified list of 9 factors extracted from, - and also the 24 most objective OQ's used for, - a FA of previously obtained data[3]. The 9 factors which accounted for > 69% of the total sensory variance in cooked beef aroma of 14 samples, were

F1 - Meaty Factor
F2 - Cooked Vegetables Factor
F3 - Toasted/Burnt Factor
F4 - Ammoniacal Factor
F5 - Oily/Fatty Factor
F6 - Fragrant Factor
F7 - Musty/Mouldy
F8 - Flat/Dull Factor
F9 - Buttery Factor

Sensory differences were evident between fractions eluting from the Tenax during the 3 separate stages of heat desorption. When these were correlated chromatographically, results showed that peaks 1-47 and their co-eluents (see Table 27, p. 122) were associated with the buttery and sweet notes of the N_2 pre-flush/ heating periods of heat desorption. In particular, butan-2,3- dione (diacetyl), but-2-en-1-al, a methylpentanol-actone and 3-hydroxybutanone (acetoin) are likely to be major contributors. Peak 49 onwards and their co-eluents (see Table 27, p.122) were associated with the meaty, charred notes predominating in the flush-on period.

Use of a globe acting as a mixing chamber enabled presentation of the aroma isolate as an entity, thus avoiding the fractionation effect just described. Sensory perception of representative cooked beef aroma using the Globe Technique validated the novel approach to sensory analysis of aroma isolates.

Chemicals which correlated with sensory differences between fractions containing the smaller and larger GC peaks respectively showed peaks 37-56 and 58-65 (see Table 12, 7) to occur exclusively in the "small peak fraction" which was scored more highly for meaty qualities during the flush-on period of heat desorption. Compounds which may have accounted for the buttery, sweet and empyreumatic notes associated with the "large peak fraction" during this heat-desorption period are

diacetyl, pentan-2-one and dimethyltrisulphide.

Correlating the chemicals and their co-eluents with meaty and burnt/ toasted GC areas, as defined by OPA, implicated the identified aldehydes (especially 3-methylbutanal), furanoids, oxazolines and thiazoles as having particular importance to meaty character of cooked beef aroma. Similarly the furanoids, thiophens, oxazolines and thiazoles identified were demonstrated to be of importance to the roasted/toasted qualities of cooked beef aroma. In particular, 2,4-dimethyl-5-ethythiazole (which on its own is known to possess a meaty and roasted aroma[137]) and which was identified in both meaty and roasted/toasted GC areas, is thus very likely to be an important contributor to roast beef aroma. It is possible that those unidentified compounds possessing meaty aromas may be mercapto-substituted furans and/ or mercapto-substituted thiophens which, when synthesised are known to possess meaty and roasted aromas[16,199], and which may be expected, but as yet have not been identified in cooked beef aroma. In the present project, meaty roast qualities were shown to be associated with relatively high boiling compounds present in trace quantities. For the first time in aroma research a rigorous statistical analysis was performed. A SRA of previously obtained chemical data onto the 9 sensory factors extracted by FA showed that the Meaty Factor could be expressed as

$$F1 = -0.754 + 0.011 \text{ alkenes} + 0.038 \text{ alcohols} + 0.027 \text{ ketones} + 0.029 \text{ furons} + 0.025 \text{ pyrazines} \quad (\bar{R}^2 = 0.812)$$

Likewise the Burnt/Toasted Factor could be expressed as

$$F3 = -0.135 + 0.003 \text{ aldehydes} + 0.018 \text{ ketones} + 0.022 \text{ furans} - 0.073 \text{ pyrroles} - 0.025 \text{ pyridines} \quad (\bar{R}^2 = 0.668.)$$

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